REPORT

of

THE COUNCIL FOR TOBACCO RESEARCH-U.S.A., Inc.

1982

Organization and Policy

The Council for Tobacco Research–U.S.A., Inc. is the sponsoring agency of a program of research into questions of tobacco use and health. It is the outgrowth of an organization formed early in 1954 by tobacco manufacturers, growers and warehousemen. Research support has been mainly through a program of grants-in-aid supplemented by contracts for research with institutions and laboratories. The Council does not operate any research facility.

The Scientific Advisory Board to The Council meets regularly to evaluate applications for research support, judging them solely on the basis of scientific merit and relevance.

The Council awards research grants to independent scientists who are assured complete scientific freedom in conducting their studies. Grantees alone are responsible for reporting or publishing their findings in the accepted scientific manner – through medical and scientific journals and societies.

WILLIAM D. HOBBS
Chairman

1982 REPORT

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THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., Inc.

THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., Inc.
110 East 59th Street, New York, N.Y. 10022

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to The Council for Tobacco Research—U.S.A., Inc.
as of December 31, 1982

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Introduction

The Council for Tobacco Research's program expanded further in 1982 in response to a growing number of solid and often quite imginative grant applications that continued to come from independent scientists. It seemed clear during the year that even with the vast amount of research done for nearly three decades into questions related to smoking and health, many in the scientific community believe important gaps in knowledge remain to be filled.

Therefore, reflecting what appears to be an increased interest in smoking and health research among scientists, the Council substantially enlarged its annual commitment. The action also reflected the Council's support for the plans and desires of its Scientific Advisory Board, which is always on the lookout for qualified investigators to study specific areas that are considered important.

Since 1954, the Council has provided $76,000,000 for its research program, which is believed to be the largest and most extensive of its kind in the world. Today, as at the outset, the program emphasizes research by independent scientists in lung cancer, cardiovascular diseases and chronic pulmonary ailments.

These funds were for 829 original grants and numerous renewals, since many of the projects were for two and three years or longer. The Council has, through the years, funded 466 researchers in 272 medical schools, hospitals and research institutions.

A measure of the Council's contribution to the advancement of scientific information is seen in the number of reports published by grantees that acknowledge Council support. That figure was 2,168 as of December 31, 1982.

The Council remains dedicated to continuance of its research effort.

Abstracts of Reports

Following are abstracts, approved by the authors, of reports on new research acknowledging support from The Council that have appeared in scientific journals since publication of the 1981 Report. The name of the grant recipient is in italics.

The abstracts are grouped under these headings: I. Cancer-Related Studies, II. The Respiratory System, III. Heart and Circulation, IV. Neuropharmacology and Physiology, V. Pharmacology and Biochemistry, VI. Immunology and Adaptive Mechanisms, VII. Epidemiology.

I. *Cancer-Related Studies*

MECHANISM OF ACTION OF BENZO[α]PYRENE AND NICOTINE ON HORMONE PRODUCTION BY RAT PITUITARY TUMOR CELLS

Although hormones have been associated with induction and progression of tumors in many experimental systems, the role of hormones in the process of initiation and progression of carcinogenesis is not clearly defined as yet. In the present attempt to understand the mechanism of action of benzo(α)pyrene (BaP), a cyclic aromatic hydrocarbon, and that of nicotine, the tobacco alkaloid, the effects of these agents on prolactin (PRL) and growth hormone (GH) synthesis by rat pituitary tumor cells in culture (GH cells) were studied. Treatment of GH cells with nicotine (0.1-300 μg/ml) neither affected the growth nor significantly altered the general pattern of hormone production in these cells. BaP at concentrations greater than 5μg/ml irreversibly inhibited the growth of these cells. The sublethal concentrations of BaP, which did not affect either (1) cell growth, or (2) amino acid transport or (3) total protein synthesis or degradation, did however inhibit specifically hormone synthesis by these cells. More interestingly, concentrations of nicotine, which did not affect either cell growth or hormone synthesis, modulated both of these cellular processes in the presence of BaP. A concentration dependent stimulation of microsomal BaP monooxygenase activity was observed in nicotine or BaP treated cells. The effects of these substances on stimulation of BaP monooxygenase activity seems to be additive. Nicotine also enhanced the association of radioactivity (presumably [^{3}H]BaP metabolites) with DNA in [^{3}H]BaP treated cells. It is concluded that nicotine by itself did not demonstrate any cytotoxic effect nor influence hormone synthesis in GH cells. However, nicotine stimulated BaP monooxygenase activity and the interaction of [^{3}H]BaP metobolites with cellular DNA and also modulated BaP induced inhibition of hormone synthesis in GH cells.

Chakrabarti, S., Hanes, S. D. and *Biswas, D. K.*

Biochemical and Biophysical Research Communications 108(2):596-603, 1982.

From the Laboratory of Pharmacology, Harvard School of Dental Medicine and Department of Pharmacology, Harvard Medical School, Boston.

EFFECTS OF SELENIUM AND SULFUR ON METABOLISM OF BENZO[α]PYRENE BY HUMAN PULMONARY ALVEOLAR MACROPHAGES

The interaction between trace metals and polycyclic acromatic hydrocarbons (PAHs) has attracted increased interest recently since some trace metals, including selenium, have been shown to decrease chemical carcinogenesis. Due to the high absorption efficiency of trace metals in the lung alveolus and the ability of pulmonary alveolar macrophages (PAMs) to metabolize PAHs, attention is centering now on the role of PAMs as a primary defense against xenobiotics. For the studies reported here, focus was on the effects of selenium (as Na_2SeO_3) and sulfur (as Na_2SO_3) on cytotoxicity and conjugation of benzo(α)pyrene (BaP) by human PAMs. Results showed that, although selenium was found to be more cytotoxic than sulfur, the cytotoxicity of both selenium and sulfur was greater for PAMs obtained by bronchopulmonary lavage from nonsmokers than for those obtained from cigarette smokers. At 1μM selenium, glutathione conjugation of BaP was enhanced in both smoker and nonsmoker PAMs. Even though selenium toxicity can be achieved in PAMs at high levels (100μM), it seems that this element may afford protection by induction of detoxification enzymes. Indeed, the lack of selenium cytotoxicity in smoker PAMs suggests a protective effect from cigarette smoke components, including the trace metals studied here.

Marshall, M. V., McLemore, T. L., *Busbee, D. L.*, Martin, R. R., and Griffin, A. C.

In: *Polynuclear Aromatic Hydrocarbons: Fifth International Symposium on Chemical Analysis and Biological Fate*, Columbus, Ohio: Battelle Press, 1981, pp. 221-230.

Other support: The Robert A. Welch Foundation and the American Cancer Society.

From the University of Texas System Cancer Center and Baylor College of Medicine, Houston; North Texas State University, Denton.

ONTOGENETIC VARIATION IN RAT LIVER, LUNG AND KIDNEY MONOOXYGENASE INDUCTION BY LOW DOSES OF BENZO(α)PYRENE AND CIGARETTE-SMOKE CONDENSATE

In previous *in vitro* studies, it has been shown that aryl hydrocarbon hydroxylase (AHH) is specifically induced in the lung and kidney of animal subjects by the inhalation of cigarette smoke, whereas cigarette smoke has no action on AHH activity in the liver. These observations were attributed to the route followed by the cigarette smoke components after inhalation. However, the paper presented here, which deals with two routes of administration, inhalation and i.p. injection, adds another parameter to the investigative process. When administered i.p., cigarette smoke condensate (CSC) and benzo(α)pyrene (BP) in low doses reached the liver before the other organs. Nevertheless, the lung was still the most sensitive organ of the three. Results showed that, in the liver and kidney, basal AHH activity (which is low in the fetus) increases rapidly after birth to reach the adult level two months later, and is only inducible by CSC and low doses of BP in unweaned rats. In the lung, however, the basal AHH activity (low in the fetus) increases abruptly at birth, peaks in five-day-old rats and then decreases slightly. Contrary to enzyme activity in other tissues, lung AHH cannot be induced in unweaned young animals. The enzyme subsequently becomes sensitive to inducing agents and is highly inducible in 90-day-old rats. Similar behavior has been shown to

occur in two other enzymes linked to cytochrome P_1450: ethoxycoumarin deethylase and ethoxyresorufin deethylase. According to the authors of this paper, the special AHH inducibility of the lung of the adult animal is a very important biological fact, and forthcoming studies should give insight into the cause and biological consequences of this phenomenon.

Van Cantfort, J. and *Gielen, J.E.*

British Journal of Cancer 44:902-910, 1981.

Other support: Fonds de la Recherche Scientifique Medicale.

From the Laboratoire de Chimie Médicale, Institut de Pathologie, Université de Liège, Liège, Belgium.

ARYL HYDROCARBON HYDROXYLASE ACTIVITY IN PULMONARY MACROPHAGES AND BLOOD LYMPHOCYTES

In the study reported here, the simultaneous analysis of (1) aryl hydrocarbon hydroxylase (AHH) induction in cultured lymphocytes and (2) *in situ* levels of AHH activity in pulmonary alveolar macrophages (PAMs) was undertaken. The study group included eight lung cancer patients with asbestos exposure, 15 non-cancer patients with asbestos exposure, 21 patients with lung cancer but without asbestos exposure, and 40 patients with neither lung cancer nor asbestos exposure. All patients were cigarette smokers who were admitted for indications of pulmonary disease. When all patient groups were compared in terms of their individual capacities for tissues to be induced, striking differences were seen between the groups. Those individuals with asbestos exposure presented increased AHH activity when compared to those without asbestos exposure when lung cancer and non-cancer groups were compared. Among cigarette smokers with neither lung cancer nor occupational asbestos exposure, 69% had relatively low AHH levels in both lymphocytes and PAMs. When AHH values were examined in asbestos-exposed smokers without lung cancer, 57% of the individuals exhibited low AHH levels in both tissues. When a simultaneous comparison was made for cigarette smokers with lung cancer but without asbestos exposure, only 36% of the individuals could be classified as having low AHH values in both lymphocytes and PAMs. Finally, when AHH values were compared for tissues obtained from asbestos-exposed lung cancer patients, the data showed that 100% of these individuals possessed high AHH activity in either lymphocytes or in PAMs, but not in both tissues. Overall, these results suggest that asbestos exposure may effect changes in a person's AHH responsiveness or total induced level in such a way as to increase that individual's susceptibility to the materials found in cigarette smoke condensate.

Snodgrass, D. R., McLemore, T. L., Teague, R. B., Wray, N. P., and *Busbee, D. L.*

CHEST 80S:42S-44S, 1981.

Other support: American Cancer Society, Veterans Administration Hospital, Houston, and the National Institutes of Health.

From the Department of Biological Sciences, North Texas State University, Denton; Department of Medicine, Baylor College of Medicine, and the Veterans Administration Hospital, Houston.

POSITIVE CORRELATION BETWEEN HIGH ARYL HYDROCARBON HYDROXYLASE ACTIVITY AND PRIMARY LUNG CANCER AS ANALYZED IN CRYOPRESERVED LYMPHOCYTES

Blood samples from 51 patients at the Veterans Administration Hospital, Houston, were collected, coded, and sent to Microbiological Associates, Bethesda, MD, where the lymphocytes were isolated and cryopreserved at −190°C before examination. At the time of assay, lymphocyte samples were simultaneously thawed, phytohemagglutinin activated, and analyzed for benz(a)anthracene-induced aryl hydrocarbon hydroxylase (AHH) levels, [^{3}H]thymidine incorporation, and reduced nicotinamide adenine dinucleotide-dependent cytochrome b, (cytochrome c) reductase activity. Determinations were made at both 96 and 120 hr in culture, and peak activities were compared among the 51 individuals who expressed such lesions as squamous cell carcinomas (22%), adenocarcinomas (14%), oat cell carcinomas (6%), chronic obstructive pulmonary disease (22%), and other nonmalignant diseases. Of the 14 highest AHH/cytochrome c activities observed, all were found in patients with primary lung cancer. Mean AHH/cytochrome c activities were 0.89 for lung cancer patients (a total of 21) and 0.47 for noncancer patients (a total of 30). No relationship was observed between AHH/cytochrome c activity and age of patient, number of cigarettes smoked, family history of cancer, location or histological type of tumor, or level of phytohemagglutinin blastogenesis ([^{3}H]thymidine cpm/cytochrome c). Although the present communication presents data which show a striking correlation between the presence of pulmonary carcinomas and high AHH levels in lymphocytes isolated from patients, whether the higher AHH levels are the cause or the result of the primary lung cancer still remains to be determined.

Kouri, R.E. *et al.* *(Microbiological Associates)*

Cancer Research 42(12):5030-5037, 1982.

Other support: American Cancer Society and the Veterans Administration Hospital, Houston.

From the Division of Toxicology and Oncology, Microbiological Associates, Bethesda, MD, Department of Biological Sciences, North Texas State University, Denton, and the Department of Medicine, Baylor College of Medicine, and Veterans Hospital, Houston.

ARYL HYDROCARBON INDUCIBILITY IS NOT ALTERED IN BLADDER CANCER PATIENTS OR THEIR PROGENY

The role of aryl hydrocarbon hydroxylase (AHH) inducibility in predisposing persons to cancer has been the subject of considerable controversy, with some reports showing an increased risk of respiratory cancer in persons with high AHH inducibility and others showing no such effect. In the present study, the possible influence of AHH on susceptibility to bladder cancer in humans was carefully investigated. AHH inducibility was measured in the cultured lymphocytes of 16 patients who were being followed after successful treatment for bladder cancer, in 53 progeny of bladder cancer

patients, and in matched controls. In both the progeny and patient populations, no evidence was found for a difference between the distribution of AHH inducibility or induced AHH activity compared to the distribution among control individuals. Thus, AHH activity or inducibility did not appear to be a major determinant of bladder cancer risk in humans.

Paigen, B. *et al. (Paigen, K.)*

International Journal of Cancer 23:312-315, 1979.

Other support: National Cancer Institute

From the Department of Molecular Biology, Roswell Park Memorial Institute, Buffalo.

MONOOXYGENASE AND EPOXIDE HYDROLASE REGULATION IN PRIMARY FETAL RAT LIVER CELL CULTURE

In this report, various lines of evidence are presented demonstrating that a cytochrome P450 comparable to that of the adult rat liver can be found in fetal hepatocyte culture, and that its appearance is strictly controlled by corticoids. The first evidence cited here shows that the level of the cytochrome P450 content in primary fetal liver cell culture is remarkably stable and does not significantly differ from that found in fetal liver used for culture preparation. Aryl hydrocarbon hydroxylase (AHH) and epoxide hydrolase (EH) activities are easily measurable in the cells and tend to decrease slightly as a function of the culture duration. High concentration of phenobarbital (PB) induces both AHH and EH activities, whereas benz (a)anthracene (BA) acts preferentially and 2,3,7,8-tetrachlorodibenzo-p-dioxin selectively on AHH activity. Trans-stilbene oxide and ethoxyquin behave as selective inducers of EH. Other evidence shows that AHH and EH activities in fetal liver cells are modified in a parallel manner by the addition of dexamethasone to the culture medium. This effect is biphasic as the enzyme activities are first inhibited and then induced when the corticoid concentration is progressively raised. The corticoid also modifies the AHH activity on a qualitative basis. In the absence of the corticoid, the enzyme activity is inhibited *in vitro* by α-naphthoflavone but not by metyrapone. Dexamethasone also modifies the induction of AHH by PB and BA, both on a qualitative and quantitative basis. Overall, on the basis of this and other evidence, it seems quite reasonable to assume that primary fetal rat liver cells in culture might constitute an interesting model for studying the physiological regulatory mechanisms of drug metabolizing enzymes.

Gielen, J. E. et al.

In: Snyder *et al.* (eds.): *Biological Reactive Intermediates-II, Part A*, New York: Plenum Publishing Corp., 1982, pp. 87-97.

Other support: Fonds National de la Recherche Scientifique.

From the Laboratoire de Chimie Médicale et de Toxicologie, Institut de Pathologie, Université de Liège, Liège, Belgium.

MULTIPLICITY OF CYTOCHROME P-450 IN PRIMARY FETAL HEPATOCYTES IN CULTURE

The perinatal period of life is a critical time for the quantitative and qualitative development of microsomal monooxygenases, and earlier observations suggest that fetal hepatocytes in culture might constitute an ideal tool for studying the perinatal regulatory mechanism of monooxygenases. While it has been known for a while that primary fetal rat hepatocytes in culture display different monooxygenase activities which can be induced by several chemical inducers, these hepatocytes were believed until now to produce only one single cytochrome P-450 species, namely the cytochrome P_1-450 (or P-448). However, it now seems possible to induce other cytochrome P-450 species in these hepatocytes, providing that they receive an appropriate hormonal treatment. In the work reported here, examination was made of the effect of dexamethasone on various monooxygenases and on the type of cytochrome P-450 supporting these enzymic activities. Three enzymes, aryl hydrocarbon hydroxylase, ethoxycoumarin deethylase and aldrin monooxygenase, were measured for this purpose. Results of this study show that the presence of dexamethasone in the culture medium produces qualitative and quantitative changes in the monooxygenase-supporting cytochrome(s) P-450. For low dexamethasone concentrations, a cytochrome P-450 is formed displaying biochemical and biophysical properties similar to those induced by phenobarbital in the adult rat liver. At higher concentrations, similar qualitative changes are observed; but a quantitative phenomenon occurs, the (cytochrome P-450)-dependent enzymic activities being also induced. Dexamethasone also has a synergistic effect in the induction of enzymic activity by the mixture of phenobarbital plus benzanthracene. The various biochemical changes induced by dexamethasone in the fetal cell cultures parallel those observed *in vivo* during the perinatal period of life. Therefore, this cell culture system may constitute an interesting model for studying the ontogenic development of liver monooxygenases.

Kremers, P., Goujon, F., De Graeve, J., Van Cantfort, J. and *Gielen, J. E.*

European Journal of Biochemistry 116:67-72, 1981.

Other support: Fonds de la Recherche Scientifique Médicale.

From the Laboratoire de Chimie Médicale, Institut de Pathologie, Université de Liège, Liège, Belgium.

DNA METHYLATION IN NORMAL AND SV40-TRANSFORMED HUMAN FIBROBLASTS

The 5-methylcytosine base content of DNA in four normal and four SV40-transformed human diploid fibroblast cultures was measured by high performance liquid chromatography (HPLC). Results show that the percent of cytosines methylated for the four normal cell lines ranged from 2.83 to 3.18, while the range for the four SV40-transformed cells was from 2.90 to 3.03. The mean for the total number of HPLC determinations was 2.94 ± 0.28 (51 determinations) for the normal cell types and 3.00 ± 0.28 (53 determinations) for the transformed lines. Thus, in contrast to other

reported studies comparing normal and oncogenically transformed cells, no apparent difference was observed in the 5-methylcytosine to cytosine base ratios in the two cell types. It is worth emphasizing that the HPLC method used here gives an absolute measure of the DNA bases. In addition, the purity of the DNA is controlled by monitoring for the presence of uracil. Other methods using radioactive label may be hindered by various different artifacts.

Diala, E. S., Plent, M. M., Coalson, D. W., and *Hoffman, R. M.*

Biochemical and Biophysical Research Communications 102(4):1379-1384, 1981.

Other support: National Institutes of Health, The United Cancer Council, Inc., The Cancer Research Coordinating Committee of the University of California, the Academic Senate, University of California, San Diego, and the Leukemia Society of America.

From the Department of Pediatrics, University of California at San Diego School of Medicine, La Jolla.

CONSTITUTIVE BEHAVIOR OF METHIONYL-tRNA SYNTHETASE COMPARED TO REPRESSIBLE BEHAVIOR OF METHIONINE ADENOSYLTRANSFERASE IN MAMMALIAN CELLS

Methionine, because of its roles in protein synthesis and in methylation, is of central importance to all cells. In the metabolic process, methionine can be used by the cell through two different pathways: (1) methionine can be converted to S-adenosylmethionine, the major methyl source for cellular transmethylation reactions and the source of the propylamine group for polyamine biosynthesis, or (2) it can be converted to methionyl-tRNA, an important intermediate in protein biosynthesis. In the paper presented here, it is reported that methionyl-tRNA synthetase, unlike methionine adenosyltransferase, behaves in a constitutive manner with respect to the concentration of methionine in the culture medium. This behavior is seen in Chinese hamster ovary cells and in normal diploid and SV40-transformed human fibroblasts. Although the kinetics of regulation of methionine adenosyltransferase and methionyl-tRNA synthetase by exogenous methionine are clearly different, the levels of the two enzymes in the human cell lines are similar.

Rubnitz, J. E., Jacobsen, S. J. and *Hoffman, R. M.*

Biochimica et Biophysica Acta 677:269-273, 1981.

Other support: National Institutes of Health, The United Cancer Council, Inc., The Cancer Research Coordinating Committee of the University of California, the Academic Senate, University of California, San Diego, and the Leukemia Society of America.

From the Department of Pediatrics, University of California at San Diego School of Medicine, La Jolla.

FOLATE POLYGLUTAMATE AND MONOGLUTAMATE ACCUMULATION IN NORMAL AND SV40-TRANSFORMED HUMAN FIBROBLASTS

In the present attempt to ascertain the role of folate polyglutamates in cell division, it seemed necessary first to starve the cells of folates and estimate their total folate requirements for growth. All four cell lines studied here, the normal human diploid foreskin fibroblast BA, the normal human fetal diploid AF2, and the SV40-transformed lines P5 and P1, showed similar kinetics for folate starvation. Sephadex G-10 gel filtration chromatography was used to measure the accumulation of folate polyglutamate and monoglutamate in all of these cell lines. After the cells had been depleted of folates, they were provided with limiting amounts of [^{3}H]-folic acid in order that the cells would accumulate only forms of folate necessary for proliferation. Both the normal and the transformed cells accumulated monoglutamate and polyglutamate forms, but by 72 hours of labeling, the transformed cells contained 3-10 times more polyglutamate then the normal cells. The growth rates for the normal and transformed cells were similar at this limiting folic acid concentration. Thus, if folate polyglutamates are more important for the proliferation of SV40-transformed cells than the normal cells, the inhibition of polyglutamate formation could possibly be an important potential target for chemotherapy.

Hoffman, R. M. et al.

Journal of Cellular Physiology 109:497-505, 1981.

Other support: National Institutes of Health, The United Cancer Council, Inc., The Cancer Research Coordinating Committee of the University of California, the Academic Senate, University of California, San Diego, and the Leukemia Society of America.

From the Department of Pediatrics, University of California at San Diego School of Medicine, La Jolla, and the Genetics Unit, Children's Service, Massachusetts General Hospital, Department of Pediatrics and Center for Human Genetics, Harvard Medical School, Boston.

DNA METHYLATION LEVELS IN NORMAL AND CHEMICALLY-TRANSFORMED MOUSE 3T3 CELLS

This investigation was undertaken to assess the effect of chemical transformation on total genomic DNA methylation as measured by high performance liquid chromatography (HPLC). In the study presented here, normal mouse embryo 3T3 cell cultures and those oncogenically transformed by the chemical carcinogens benzo(a)pyrene and methylcholanthrene were analyzed by HPLC to determine the 5-methycytosine to cytosine base ratios in their total genomic DNA. Results showed that the mean for 10 HPLC determinations of the normal 3T3 cells was 2.87% of cytosines methylated with a standard deviation of ± 0.58, while that for the benzo(a)pyrene-transformed 3T3 cells and the methylcholanthrene-transformed 3T3 cells was $2.88\% \pm 0.31$ (13 determinations) and $2.81\% \pm 0.18$ (15 determinations), respectively. These results led to the conclusion that there is no real difference in the extent of total genomic DNA methylation between normal and chemically-transformed 3T3 cells when measured by HPLC.

Diala, E. S. and *Hoffman, R. M.*

Biochemical and Biophysical Research Communications 104(4):1489-1494, 1982.

Other support: National Institutes of Health, The United Cancer Council, Inc., The Cancer Research Coordinating Committee of the University of California, the Academic Senate, University of California, San Diego, and the Leukemia Society of America.

From the Department of Pediatrics, University of California at San Diego School of Medicine, La Jolla.

METHIONINE DEPENDENCE IN CANCER CELLS—A REVIEW

Methionine dependence occurs in a large number and wide variety of cancer cells and does not seem to be a random component of the transformed phenotype. Definition of methionine dependence states that it is a defect found in many cancer cell lines that inhibits their growth in culture when methionine is replaced by its immediate precursor, homocysteine, in the culture medium. Normal cultured cells do not have this defect. This report lists the diverse and large number of animal and human cancer lines that are methionine-dependent, and critically reviews the cell biology and methionine biochemistry of the phenomenon.

Hoffman, R. M.

In Vitro 18(5):421-428, 1982.

Other support: National Institutes of Health, The United Cancer Council, Inc., The Cancer Research Coordinating Committee of the University of California, the Academic Senate, University of California, San Diego, and the Leukemia Society of America.

From the Department of Pediatrics, University of California at San Diego School of Medicine, La Jolla.

HYPOMETHYLATION OF HELA CELL DNA AND THE ABSENCE OF 5-METHYLCYTOSINE IN SV40 AND ADENOVIRUS (TYPE 2) DNA: ANALYSIS BY HPLC

In the study presented here, methylation of the purified virion DNA of both SV40 and adenovirus (type 2) was measured by high performance liquid chromatography (HPLC) and compared to their hosts, African green monkey kidney cells and HeLa cells, respectively. In SV40 DNA, as much as 12 nanomoles of cytosine has been measured without concomitant detection of m^5Cyt. SV40 contains 27 CpG pairs, which is the usual methylation site. If all CpG pairs were methylated, this would yield a significant 1.3% methylation of total cytosines; however, in the virion DNA studied here, none seemed to be methylated. Also, as with SV40, m^5Cyt was not present in the

elution pattern of virion adenovirus (type 2) DNA bases. Overall, essentially no 5-methylcytosine was detected in either viral DNA. Implications for viral gene regulation by methylation are discussed here. In comparison with normal human cell DNA methylation levels, HeLa cell DNA methylation is reduced significantly.

Diala, E. S. and *Hoffman, R. M.*

Biochemical and Biophysical Research Communications 107(1):19-26, 1982.

Other support: National Institutes of Health.

From the Department of Pediatrics, University of California at San Diego School of Medicine, La Jolla.

REDUCED AVAILABILITY OF ENDOGENOUSLY SYNTHESIZED METHIONINE FOR *S*-ADENOSYLMETHIONINE FORMATION IN METHIONINE-DEPENDENT CANCER CELLS

Methionine (Met) dependence—*i.e.*, the inability of cultured cells to grow when Met is replaced by its immediate precursor homocysteine (Met^-Hcy^+ medium)—is a frequent component of the oncogenically transformed phenotype. Normal cells, on the other hand, grow in this medium. There have been reports [Hoffman, R. M. & Erbe, R. W. (1976) *Proc. Natl. Acad. Sci.* USA 73, 1523-1527; Hoffman, R. M., Jacobsen, S. J. & Erbe, R. W. (1978) *Biochem. Biophys. Res. Commun.* 82, 225-234] of normal or higher rates of Met biosynthesis in Met-dependent cells and a postulation that Met-dependent cells are deficient in utilization of endogenously synthesized Met as opposed to exogenously supplied Met. To answer the critical question of what biochemical reaction(s) requires preformed Met in Met-dependent cells, we labeled cells with Met-free [^{35}S] Hcy or [^{35}S] Met and determined the levels of Met, S-adenosylmethionine (AdoMet), and S-adenosylhomocysteine (AdoHcy). We report here experiments that demonstrate that Met-dependent cells synthesize a normal amount of endogenously synthesized Met and are deficient in utilizing this Met for AdoMet synthesis. In contrast, exogenously supplied Met is utilized normally for AdoMet biosynthesis. The ratio of AdoMet to AdoHcy is low in Met-dependent cells growing in Met^-Hcy^+ medium. We determined that the low AdoMet/AdoHcy ratio probably limits growth of Met-dependent cells in Met^-Hcy^+ medium.

Coalson, D. W., Mecham, J. O., Stern, P. H., and *Hoffman, R. M.*

Proceedings of the National Academy of Sciences of the United States of America 79:4248-4251, 1982.

Other support: National Institutes of Health, The University Cancer Council, Inc., The Cancer Research Coordinating Committee of the University of California, the Academic Senate, University of California at San Diego, and the Leukemia Society of America.

From the Department of Pediatrics, University of California at San Diego School of Medicine, La Jolla.

VASCULAR INVASION OF CARTILAGE: CORRELATION OF MORPHOLOGY WITH LYSOZYME, GLYCOSAMINOGLYCANS, PROTEASE, AND PROTEASE-INHIBITORY ACTIVITY DURING ENDOCHONDRAL BONE DEVELOPMENT

Although it is well known that cartilage vascularization is a prerequisite for bone differentiation, the precise mechanisms of this vascularization are incompletely understood. In the present attempt to study these changes, demineralized bone matrix prepared from rat diaphyses was transplanted subcutaneously into bilateral sites in the thoracic region of 28- to 35-day old male Long-Evans strain rats; the day of transplantation was designated as Day 0. While using the matrix-induced endochondral bone differentiation as a model system, changes in the levels of lysozyme, patterns of glycosaminoglycans, and activities of proteases and protease inhibitors were studied during matrix-induced cartilage, bone, and bone marrow development. The morphological transitions were correlated with the biochemical parameters. Results showed that there was a peak in lysozyme content on Day 3, during mesenchymal cell proliferation, followed by a decline during endochondral bone formation. The lysozyme levels increased again and attained maximal values during hematopoiesis on Day 21. Protease-inhibitory activity was maximal during chondrogenesis and diminished during osteogenesis. Protease activity was maximal on Day 3 during mesenchymal cell proliferation and was apparently present as an enzyme-inhibitor complex. Vascularization and bone formation were accompanied by an increase in protease activity. Chondroitin-4-sulfate was the predominant glycosaminoglycan detected in the matrix-induced cartilage and bone.

Reddi, A. H. and *Kuettner, K. E.*

Developmental Biology 82:217-223, 1981.

Other support: National Institutes of Health.

From the Laboratory of Biological Structure, National Institute of Dental Research, National Institutes of Health, Bethesda, MD., and the Departments of Orthopedic Surgery and Biochemistry, Rush-Presbyterian-St. Luke's Medical Center, Chicago.

RESISTANCE OF CARTILAGE TO INVASION

In this extensive and carefully worked-out chapter, a simplified concept of the invasive process is introduced, and a unique sequence of events occurring during physiologic (endothelial cells) and pathologic (osteosarcoma cells) invasion is described. The term invasion, as used here, is defined as the passing, interpenetration, or infiltration of cells into adjacent tissues. The events that characterize this process have been studied *in vitro* in a combined organ-cell culture system consisting of mamalian hyaline cartilage and TE-85 osteosarcoma cells. Cartilage was able to resist the invasion by osteosarcoma cells in the organ-cell culture system. Osteosarcoma cells forced to grow in direct contact with the cut surfaces of articular hyaline cartilage piled up to several cell layers, but were unable to penetrate the cartilaginous matrix. *In vivo*, similar restrictions of cartilage to tumor cell invasion have been reported in primary and metastatic tumors. Also, cartilage is thought to be responsible for the slow invasion of carcinoma cells into the walls of the larynx. Specific studies reported here show that hyaline cartilage, mildly extracted with various salt solutions, is readily infiltrated by osteosarcoma cells in the organ-cell culture system. These findings led to

the conclusion that cartilage contains extractable matrix compounds that inhibit invasion in an experimental system. When these diffusable and extractable substances were further studied, it was found that the inhibition of osteosarcoma cell proliferation was caused by molecules with a molecular weight of less than 50,000 daltons. From this anti-invasive factor of the cartilage extract, a protease inhibitor was identified that has the ability to inhibit mamalian collagenase, including that elaborated by osteosarcoma cells and endothelial cells. These experimental data led to the hypothesis that invasion of either tumor or endothelial cells depends on proteolytic (collagenolytic) enzyme activities. Other studies along these lines have been instituted to follow the invasiveness and proliferation of bladder cancer.

Kuettner, K. E. and Pauli, B. U.

In: Gilbert, H. A., Weiss, L. and Monsen, D. C. G. (eds.): *Bone Metastasis,* Boston: G. K. Hall Medical Publishers, 1981, pp. 131-165.

Other support: National Institutes of Health.

From the Departments of Orthopedic Surgery, Pathology and Biochemistry, Rush-Presbyterian-St. Luke's Medical Center, Chicago.

ANTIINVASION FACTOR MEDIATES AVASCULARITY OF HYALINE CARTILAGE

To test an antiinvasion factor (AIF) hypothesis of the resistance of cartilage to vascular invasion, a novel *in vitro* system was studied that employed bovine articular cartilage as a growth surface for normal heparin-stimulated endothelial cells. In this study, cells were tested for their ability to invade the matrix of viable and devitalized extracted cartilage as monitored by thin-section electron microscopy. The growth behavior of cells on devitalized extracted cartilage was examined in the presence and absence of cartilage-derived, extractable AIF in the culture medium. Whereas normally viable articular cartilage is a poor growth surface for endothelial cells, the endothelial cells studied here, in contrast, grew as contact-inhibited monolayers of flattened cells on the surfaces of extracted cartilage. The cells were separated from the cartilage matrix by abundant basal lamina. There were a few microvilli at the basal plasma membrane, but there was no degradation or penetration of the collagenous matrix of extracted cartilage. However, when endothelial cells were stimulated by heparin, they assumed a polyhedral shape and penetrated the extracted cartilage with numerous microvilli and some cytoplasmic processes. This penetration of the collagenous matrix was associated with tissue rarefaction and degradation of collagen fibers. Importantly, however, this invasion of heparin-stimulated endothelial cells was abolished when low concentrations of cartilage-derived AIF were added to the culture medium. These data provide evidence that the resistance of hyaline cartilage to endothelial cell invasion is regulated in part by tissue derived proteinase inhibitors and an antiproliferative activity directed against endothelial cells.

Kuettner, K.E. et al

Seminars in Arthritis & Rheumatism 11:67-69, 1981.

Other support: National Institutes of Health.

From Rush Medical College, Chicago.

CHARACTERIZATION OF ADULT BOVINE ARTICULAR CHONDROCYTES IN CULTURE

Articular cartilage slices, obtained from 18-mo-old bovine metacarpophalangeal joints, were used as source material for this descriptive article of chondrocytes in culture. After sequential digestion and filtration, cells were plated in either tissue culture dishes or roller bottles. Chondrocytes fixed in buffered glutaraldehyde containing 0.1% ruthenium red, were examined by light and transmission electron microscopy. Collagen type determination of ^{3}H-proline-labeled proteins isolated from cultures were performed by electrophoretic and CNBr peptide analysis. Biosynthesis of proteoglycans was measured by $^{35}SO_4$ incorporation into macromolecules extracted under dissociative conditions. Examination showed that isolated chondrocytes prior to culture were typically rounded with scant territorial matrix, which could be removed by mild trypsinization. Throughout the progression of the cultures, phenotypic alterations were not observed. Electrofluorographs of collagen that was extracted from ^{3}H-proline-labeled cultures after mild pepsin digestion showed 1 band in the position of the α1 chain; an α2 chain could not be detected. Cyanogen bromide peptide analysis confirmed that the major radioactive peptides comigrated with unlabeled peptides obtained from type II collagen; type I collagen was not detectable in these cultures. Proteoglycan aggregate was extracted from both culture dishes and roller bottle cultures, under associative conditions. There were differences noted in this experiment suggesting that the roller culture cell-associated matrix may have a greater degree of organization than that grown in standard tissue culture dishes. These same data also indicate that articular chondrocytes grown in mass roller cultures are capable of synthesizing a phenotypically stable, tissue-like matrix *in vitro*.

Kuettner, K. E. et al.

Seminars in Arthritis & Rheumatism 11:101-103, 1981.

Other support: National Institutes of Health.

From Rush Medical College and the University of Illinois Dental School, Chicago.

REGULATION OF TUMOR INVASION BY CARTILAGE-DERIVED ANTI-INVASION FACTOR *IN VITRO*

Mammalian cartilage is highly resistant to invasion by tumor cells. This resistance was studied here with the use of a novel *in vitro* culture system. Articular cartilage obtained from fresh metacarpophalangeal joints of preadolescent bovines was used as a growth surface for human TE-85 osteosarcoma cells and foreskin fibroblasts. Cartilage disks formed the bottoms of stainless-steel cylinders, providing closed growth chambers for these cells. Both invasive osteosarcoma cells and normal fibroblasts were unable to penetrate viable, unextracted cartilage during a 2-week culture period. When cartilage was devitalized by freezing and thawing, the tissue remained resistant to invasion. Cartilage, extracted with either 1 or 3 M guanidine hydrochloride, was invaded by osteosarcoma cells, but not by control fibroblasts. Invasion by osteosarcoma cells into salt-extracted cartilage was abolished when low concentrations of a cartilage-derived, anti-invasion factor were added to the culture medium. These data

provided evidence that the resistance of cartilage to tumor invasion is regulated in part by tissue-derived proteinase inhibitors.

Pauli, B. U., Memoli, V. A. and *Kuettner, K. E.*

Journal of the National Cancer Institute 67(1):65-73, 1981.

Other support: National Cancer Institute.

From the Departments of Pathology, Biochemistry and Orthopedic Surgery, Rush Medical College, Rush-Presbyterian-St. Luke's Medical Center, Chicago.

IN VITRO DETERMINATION OF TUMOR INVASIVENESS USING EXTRACTED HYALINE CARTILAGE

In this attempt to determine whether salt-extracted cartilage could be used as a test connective tissue for *in vitro* discrimination between noninvasive and invasive tumor cell lines, a novel *in vitro* method was devised which used salt-extracted, bovine articular cartilage as an experimental growth surface for both normal bladder epithelial cells and noninvasive, invasive, and metastatic carcinoma cell lines derived from chemical carcinogen-induced tumors of the rat urinary bladder. As monitored by thin-section electron microscopy, salt-extracted cartilage was readily penetrated by the invasive and metastatic rat bladder carcinoma cell lines. The metastatic cell line could be differentiated from the invasive, nonmetastatic cell line by its greater depth of invasion. In contrast, noninvasive carcinoma cells as well as normal bladder epithelial cells lacked the capacity to erode and penetrate the extracted matrix of the articular cartilage. Using these defined cell lines, salt-extracted cartilage can be used to reproducibly discriminate between carcinomas having different invasive potentials. This assay system may have diagnostic application for the *in vitro* staging of tumors.

Pauli, B. U., Memoli, V. A. and *Kuettner, K. E.*

Cancer Research 41:2084-2091, 1981.

Other support: National Institutes of Health.

From the Departments of Pathology, Biochemistry, and Orthopedic Surgery, Rush Medical College and Rush College of Health Sciences, Rush-Presbyterian-St. Luke's Medical Center, Chicago.

A FAMILIAL AGGREGATION OF PANCREATIC CANCER: AN IN VITRO STUDY

Pancreatic cancer, with its obscure etiology and difficult early diagnosis, presents a medical problem of staggering proportions. In the *in vitro* study presented here, a family was identified in which four individuals manifested pancreatic cancer verified through two generations. Cell cultures from split-thickness skin biopsy specimens were obtained for 24 members from three generations (17 bloodline relatives, seven family members by marriage) as well as ten nonfamily normal subjects, none with a family history of solid tumors. One of the constant features of human monolayer dermal cultures has been diploidy. On the other hand, hyperdiploidy, other than

tetraploidy, has been rarely observed in cultures from normal subjects without a family history of solid tumors. In this study, however, increased *in vitro* hyperdiploidy was observed in eight of the 17 family members tested. In the long run, aggregates of pancreatic cancer in families such as this one, and the occurrence of pancreatic cancer in some autosomal dominant cancer syndromes have added credence to the relevancy of a genetic component(s) in a fraction of pancreatic cancers. In an important way, this study has demonstrated the potential for combining detailed family data with recognition of *in vitro* biomarkers for cancer proneness as an approach to the comprehension of carcinogenesis in pancreatic cancer.

Danes, B. S. and *Lynch, H. T.*

JAMA 247(20):2798-2802, 1982.

Other support: National Institutes of Health, Danes Medical Research Fund, Cornell University Medical College, and Zemurray Foundation.

From the Laboratory for Cell Biology, Department of Medicine, Cornell University College, New York, and the Department of Preventive Medicine/Public Health, Creighton University School of Medicine, Omaha.

GENETIC/EPIDEMIOLOGICAL FINDINGS IN A STUDY OF SMOKING-ASSOCIATED TUMORS

In this genetic/epidemiological study, family histories of cancer were obtained via personal interviews from consecutively ascertained cancer patients who were under evaluation in one of two University Oncology Clinics in Nebraska. Included in the series were 147 breast cancer probands, 85 colon cancer probands, 88 lung cancer probands, and 111 probands with other cancers that have been reported to be associated with cigarette smoking (carcinoma of the oral cavity, esophagus, pancreas and urinary bladder). Smoking histories of probands and their relatives were obtained for an overlapping series of 60 lung cancer probands and 78 probands with other smoking-associated tumors. Findings revealed that, although a significant cohort effect was observed with respect to smoking habits for both relatives of lung cancer probands and for relatives of probands with other smoking-associated tumors, a corresponding trend for lung cancer frequency was observed only for relatives of lung cancer probands. This result suggests the importance of host factors in combination with environmental exposures in determining lung risk. A cohort trend for lung cancer was also apparent among relatives of breast cancer probands, but not for relatives of colon cancer probands, suggesting the possibility of an intrinsic association between carcinomas of the breast and lung. It seems reasonable that further elucidation of host factor susceptibility in lung cancer may have important etiological and preventive implications.

Lynch, H. T. et al.

Cancer Genetics and Cytogenetics 6:163-169, 1982.

From the Institute for Familial Cancer Management and Control, Inc., Departments of Preventive Medicine/Public Health, Surgery, and Pathology, Creighton University School of Medicine, Omaha.

LOW SERUM IgA IN A FAMILIAL OVARIAN CANCER AGGREGATE

Although familial ovarian cancer is increasingly recognized, consistent biomarker associations that correlate with its risk have remained elusive. In this paper, however, a family is described that is characterized by excessive occurrences of ovarian carcinoma transmitted in a pattern consistent with an autosomal dominant factor. This family is unique in that identical twin sisters each had verified ovarian cancer and each had a daughter with the same lesion. Upon familial investigation, 14 of 45 individuals tested has serum IgA levels below the 95% range. Careful consideration of these results showed that low serum IgA levels were found to segregate in a sufficient number of individuals from this family to suggest that this may be a genetically determined immune defect etiologically integral to cancer susceptibility. While the putative role of IgA in pathogenesis remains elusive still, it seems apparent that cancer-prone families should be thoroughly investigated for further elucidation of these phenomena.

Schuelke, G. S., *Lynch, H. T.*, Lynch, J. F., Fain, P. R., and Chaperon, E. A.

Cancer Genetics and Cytogenetics 6:231:236, 1982.

Other support: Elsa U. Pardee Foundation.

From the Departments of Medical Microbiology and Preventive Medicine/Public Health, Creighton University School of Medicine, Omaha.

NATURAL HISTORY OF HEREDITARY CANCER OF THE BREAST AND COLON

Anecdotal reports have suggested that survival characteristics of hereditary forms of cancer may differ from their sporadic counterparts. To test this possibility, a review of disease-free survival was undertaken by evaluating the affected members of hereditary colon and breast cancer families within the extensive Creighton University Oncology Center familial cancer resources. In the study presented here, the natural history of 106 patients from 18 families manifesting hereditary breast cancer syndromes and of 117 affected patients from 20 families manifesting nonpolyposis hereditary colon cancer was evaluated. Findings were compared with the American College of Surgeons (ACS) long-term audits for breast and colon cancer respectively. The cardinal features of hereditary cancer were observed within the study group, including: (1) a significant younger age of onset (49 years, breast; 46 years, colon); (2) an excess of proximal lesions in the hereditary colon series (49%); and (3) an excess of bilaterality in the hereditary breast cancer patients. The clinical stage at presentation was similar for the hereditary and ACS audit patients. Five-year survival was significantly improved for both hereditary cancer populations as compared to the ACS audits (67% hereditary breast cancer and 52% nonpolyposis hereditary colon cancer). Improved survival in hereditary colon and breast cancer patients may have a bearing on the design of future clinical protocols.

Albano, W. A., Recabaren, J. A., *Lynch, H. T.*, Campbell, A. S., Mailliard, J. A., Organ, C. H., Lynch, J. F., and Kimberling, W. J.

Cancer 50(2):360-369, 1982.

Other support: National Cancer Institute.

From the Institute for Familial Cancer Management and Control, Inc., Creighton University School of Medicine, Omaha.

NEOPLASTIC TRANSFORMATION OF RABBIT CELLS BY MURINE SARCOMA VIRUSES

While rabbit cells have been widely used for isolation and replication of a variety of retroviruses, they have seldom been used for transformation studies with murine sarcoma viruses (MSV). In this paper, however, neoplastic transformation of rabbit cells by Kirsten MSV (Ki-MSV), the Ki-MSV pseudotype of baboon endogenous virus (Ki-MSV [BaEV]) and the Moloney-MSV pseudotype of feline leukemia virus (M-MSV [FeLV]) is reported. Rabbit cells can be readily transformed by KiMSV, Ki-MSV(BaEV) and M-MSV(FeLV). Rabbit cells transformed by Ki-MSV and M-MSV-(FeLV) were found to be virus producers, whereas those transformed by Ki-MSV(BaEV) were nonproducers (NP). The NP cells were obtained by simply infecting rabbit cells with Ki-MSV(BaEV) and subculturing the infected cells. Although the morphologically altered NP cells did not produce infectious virus or murine leukemia virus antigen, they did contain a rescuable MSV genome. All of the transformed cells formed colonies in soft agar, grew to high saturation densities and produced tumors when transplanted into nude mice. This report is the first one of successful tumor induction by virus-transformed rabbit cells. The ability to produce sarcomas in rabbits by Ki-MSV and M-MSV(FeLV)-transformed cells should provide a useful additional model for studying chemo/immunotherapy as well as immunoprevention of rabbit cancers.

Rhim, J. S., Bedigian, H. G. and Fox, R. R. (*Meier, H.*)

International Journal of Cancer 30:365-369, 1982.

Other support: National Science Foundation and the National Institutes of Health.

From the Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, MD, and The Jackson Laboratory, Bar Harbor, ME.

II. *The Respiratory System*

ELASTIN BIOSYNTHESIS IN CHICK EMBRYONIC LUNG TISSUE. COMPARISON TO CHICK AORTIC ELASTIN

In this biochemical study, the synthesis of elastin was followed in chick embryonic lung and compared to that seen in embryonic aortic tissue. Messenger ribonucleic acid (mRNA) was isolated from both lung and aortic tissues and translated in an mRNA-dependent rabbit reticulocyte lysate. The results demonstrate that both tissue RNA preparations direct the synthesis of two elastin proteins possessing molecular weights of 70 000 and 73 000, which are immunoprecipitable with antibody directed against chick aortic tropoelastin. Organ culture of embryonic lungs and aortas followed by extraction of the [^{3}H]valine-labeled proteins with urea in the presence of reducing

and alkylating reagents revealed the presence of two immunoreactive elastin proteins similar to those synthesized in the cell-free system. Limited tryptic and chymotryptic peptide mapping of the two elastin proteins synthesized in aortic organ cultures revealed a strong homology between the proteins with only minor detectable differences. Automated sequencing of [^{3}H]valine-labeled tropoelastin 70 000-dalton species) isolated from aorta and lung organ cultures demonstrated identical positions of valine residues in the NH_2-terminal region of both proteins. These results demonstrate that elastin synthesis in two unique embryonic tissues involves the production of two distinct polypeptide chains, referred to in this paper as tropoelastin a and b. Also, the 70 000-dalton protein (tropoelastin b) is similar in electrophoretic behavior to conventional tropoelastin and appears to be identical in aortic and lung tissues as judged by amino acid analyses, electrophoretic migration, high-pressure liquid chromatography, and automated sequence analyses.

Foster, J. A. et al.

Biochemistry 20(12):3528-3535, 1981.

Other support: National Institutes of Health and the National Foundation March of Dimes.

From the Department of Biochemistry, University of Georgia, Athens.

DIFFERENTIAL EXPRESSION OF AORTIC AND LUNG ELASTIN GENES DURING CHICK EMBRYOGENESIS

This quantitative study addresses the question of whether or not the ratio of tropoelastin b to a changes during embryonic development. To measure this, the rates of tropoelastin a and b synthesis were determined in short-term organ culture. The results demonstrated that in lung tissue the ratio of the two tropoelastins remained essentially constant. Each of the tropoelastins comprised 50% of the total elastin synthesis. In the aortic tissue, however, tropoelastin b represented 70% of the total elastin in the 11- to 13 day embryos and increased to 91% by Day 16. These observations seen in the organ culture system were paralleled in measurements of functional mRNAs coding for the two proteins. Measurements of functional tropoelastin mRNAs coding for the two proteins. Measurements of functional tropoelastin mRNAs from both lung and aortic tissues were performed in an mRNA-dependent rabbit reticulocyte lysate system. Although the changes in the abundance of the tropoelastin mRNAs revealed the same trend as that seen in the organ culture data, the magnitude of the tropoelastin b to a ratio in the aortic culture was twice that determined in the cell-free translation of aortic mRNAs. The most important outcome of this study was the finding that the ratios of the rates of synthesis of tropoelastin b to a as well as the amount of functional mRNA coding for these proteins differ with development as well as with tissue. Specifically, the data obtained from both cell-free translations and organ culture experiments demonstrate that there is a differential expression of elastin genes during aorta development which is significantly different from that found in developing lung.

Barrineau, L. L., Rich, C. B., Przybyla, A. and *Foster, J. A.*

Developmental Biology 87:46-51, 1981.

Other support: National Institutes of Health and the National Foundation March of Dimes.

From the Department of Biochemistry, University of Georgia, Athens.

A SURVEY OF SENSITIVE TECHNIQUES FOR EXAMINING THE *IN VITRO* SYNTHESIS OF ELASTIN

Five sensitive techniques for identification, quantitation, and characterization of soluble elastin increases are described and elaborated upon in this paper. All of these methods are applicable to picomolar amounts of tropoelastin generally encountered in *in vitro* systems. These techniques are: 2-Dimensional Gel Electrophoresis, Quantitation of Tropoelastin, Immunoprecipitation, Microsequencing of *In Vitro* Labeled Tropoelastin, and Peptide Mapping. The techniques described here provide the necessary sensitivity for describing, both directly and indirectly, the presence of tropoelastins synthesized in *in vitro* systems.

Foster, J. A. et al.

Connective Tissue Research 8:259-262, 1981.

Other support: National Institutes of Health.

From the Department of Biochemistry, University of Georgia, Athens.

EVIDENCE FOR *IN VIVO* INTERNALIZATION OF HUMAN LEUKOCYTE ELASTASE BY ALVEOLAR MACROPHAGES

Although interaction with alpha$_1$-proteinase inhibitor (α_1PI) is considered to be the major mechanism for the inactivation and clearance of potentially harmful proteases, such as leukocyte elastase, from the lung, recent evidence has suggested that direct binding and internalization of human leukocyte elastase (HLE) by alveolar macrophages may represent an alternative route for the inactivation and elimination of this lung-damaging protease. In the work reported here, cell lysates from cultured human alveolar macrophages, derived from the lungs of cigarette smokers, were shown to contain detectable amounts of an elastinolytic enzyme. Although particulate elastin was solubilized only after prolonged incubations, lysates readily hydrolyzed t-BOC-alanyl-p-nitrophenol-ester. Hydrolysis of the latter substrate was inhibited by the leukocyte elastase site-specific inhibitor, N-ac-(ala)$_4$-chloromethyl ketone. In addition, radioimmunoelectrophoresis of concentrated alveolar macrophage lysates, previously incubated with [^{3}H]diisopropyl-phosphofluoridate (DFP), revealed the presence of DFP binding material that comigrated with inactivated HLE. It seems, therefore, that the clearance of HLE by alveolar macrophages may represent a significant route for the removal of this enzyme from the lung.

White, R. *Janoff, A.*, Gordon, R., and Campbell, E.

American Review of Respiratory Disease 125(6):779-781, 1982.

Other support: National Heart, Lung and Blood Institute.

From the Department of Pathology, State University of New York at Stony Brook, Stony Brook; Department of Pathology, Mt. Sinai School of Medicine, New York; and the Department of Medicine, Washington University School of Medicine, St. Louis.

SYNTHETIC ELASTASE INHIBITORS AND THEIR ROLE IN THE TREATMENT OF DISEASE

Proteases (protein hydrolyzing enzymes) are involved in many important natural biological processes; they are also thought to be involved in many different diseases. One such disease is pulmonary emphysema, which seems to result from an imbalance between proteases released from human leucocytes and the proteases' inhibitors. Since much evidence has accumulated showing that proteolysis of lung elastin leads to the development of emphysema, it seems evident that selective elastase inhibitors could be used in the treatment of this disease. The inhibitors used could be natural inhibitors such as α_1-proteinase inhibitor itself isolated from blood fractionation, or they could be synthetic materials. Work done in the investigators' laboratory to date has shown that synthetic elastase inhibitors have considerable potential for the treatment of emphysema. Two peptide chloromethyl ketone elastase inhibitors Ac-Ala-Ala-Pro-AlaCH$_2$Cl and Suc-Ala-Pro-ValCH$_2$Cl have been shown to significantly diminish the extent of experimental-elastase-induced emphysema in hamsters. MeO-Suc-Ala-Ala-Pro-ValCH$_2$Cl has been shown to be orally active in providing protection against induced emphysema in rats. While there are still questions about whether such reactive alkylating agents could be used in the treatment of emphysema in man, the animal studies have shown that elastase inhibitors can be used to treat emphysema. Therefore, based on these studies, it seems that the overall prospects for the development of a synthetic elastase inhibitor for use in humans in the near future are quite good.

Powers, J. C. *et al. (Travis, J.)*

In: Rich, D. H. and Gross, E. (eds.): *Peptides: synthesis - structure - function, proceedings of the seventh American peptide symposium*, Rockford, IL: Pierce Chemical Company, 1981, pp, 391-399.

Other support: National Institutes of Health.

From the School of Chemistry, Georgia Institute of Technology, Atlanta.

PROTEOLYTIC ENZYMES AND THEIR ACTIVE-SITE-SPECIFIC INHIBITORS: ROLE IN THE TREATMENT OF DISEASE

Proteases (protein-hydrolyzing enzymes or proteolytic enzymes) have been known for over 100 years since trypsin was first isolated from pancreatic juice by Kühne. For most of the period since their discovery, proteases were thought to be involved only in digestion. However, in the last decade, proteases have been shown to be involved in many other important physiological processes, such as fertilization, coagulation, and the immune response. Outside of their normal environment, proteases can be extremely destructive, but natural human plasma inhibitors inhibit most proteases that escape. Imbalance in protease-protease inhibitor systems can lead to a number of diseases of which pulmonary emphysema is one well-characterized example. This disease results when the protease elastase attacks elastin, the major elastic protein in the lung. Considerable effort has been devoted to the synthesis of inhibitors of proteolytic enzymes such as elastase for possible therapeutic use. In the future, specific and selective synthetic protease inhibitors should be useful for treating specific diseases that range from the common cold to chronic disorders such as emphysema.

Powers, J. C. *(Travis, J.)*

In: Feenay, R. E. and Whitaker, J. R. (eds.): *Advances in Chemistry Series, No. 198. Modification of Proteins,* Washington, D.C.: American Chemical Society, 1982, pp. 347-367.

Other support: National Institutes of Health.

From the School of Chemistry, Georgia Institute of Technology, Atlanta.

SPECIFICITY AND REACTIVITY OF HUMAN LEUKOCYTE ELASTASE, PORCINE PANCREATIC ELASTASE, HUMAN GRANULOCYTE CATHEPSIN G, AND BOVINE PANCREATIC CHYMOTRYPSIN WITH ARYLSULFONYL FLUORIDES: DISCOVERY OF A NEW SERIES OF POTENT AND SPECIFIC IRREVERSIBLE ELASTASE INHIBITORS

The discovery of some potent and specific inhibitors for porcine pancreatic (PP) elastase, human leukocyte (HL) elastase, and chymotrypsin Aα is reported here. In particular, the reactivity and specificity of a series of substituted benzenesulfonyl fluoride with HL elastase, cathepsin G, PP elastase, and bovine chymotrypsin Aα are described. Benzenesulfonyl fluorides with 2-fluoroacyl substituents were found to be potent and specific inhibitors of elastase. HL elastase was inhibited most rapidly by $2-(CF_3CF_2CONH)-C_6H_4SO_4F$. PP elastase was most rapidly inhibited by $2-(CF_3CONH)-C_6H_4SO_2F$. The $2-(CF_3CF_2CF_2CONH)$ and $2-(CF_3SNH)$ derivatives were quite selective for HL elastase and inhibited PP elastase, cathepsin G, and chymotrypsin Aα quite slowly. A specific and potent chymotrypsin inhibitor $(2-(Z\text{-}Gly\text{-}NH)-C_6H_4SO_2F)$ was also discovered. In this paper, a model for the elastase inhibition reaction is proposed which involves interaction of the fluoroacyl group of the inhibitor with the primary substrate recognition site S_1 of the enzyme. Overall, the results of this study demonstrate that it is practically possible to construct simple organic molecules which are specific inhibitors of HL elastase, PP elastase, or chymotrypsin.

Yoshimura, T. *et al. (Travis, J.)*

The Journal of Biological Chemistry 257(9):5077-5084, 1982.

From the School of Chemistry, Georgia Institute of Technology, Atlanta.

FORMATION OF A STABLE COMPLEX BETWEEN HUMAN PROELASTASE 2 AND HUMAN α_1-PROTEASE INHIBITOR

The studies reported here were aimed at clarifying the nature of the α_1-protease inhibitor (PI)-bound immunoreactive elastase 2 in normal plasma by investigating the interaction of proelastase 2 with plasma *in vitro*. Results showed that the major proelastase 2 binding factor in human plasma is α_1-PI, and that proelastase 2 reacts directly with α_1-PI via a partial active site, in a manner similar to that of active endopeptidases. When proelastase 2 was incubated for 16 h at 25°C with 0.1 M diisopropylfluorophosphate, 0.8 mol of inhibitor was incorporated/mol of zymogen. The product no longer

yielded elastase 2 following incubation with bovine trypsin. The diisopropylphosphoryl-proelastase 2 was not able to form a complex with α_1-PI, suggesting that the potential active site serine residue in the zymogen is required for complex formation with α_1-PI.

Largman, C., Brodrick, J. W., *Goekas, M. C.*, Sischo, W. M., and Johnson, J. H.

The Journal of Biological Chemistry 254(17):8516-8523, 1979.

Other support: Medical Research Service of the Veterans Administration.

From the Enzymology Research Laboratory, Martinez Veterans Administration Medical Center, Martinez, CA, and the Department of Internal Medicine, University of California School of Medicine, Davis.

POTENTIAL MECHANISM OF EMPHYSEMA: α_1-PROTEINASE INHIBITOR RECOVERED FROM LUNGS OF CIGARETTE SMOKERS CONTAINS OXIDIZED METHIONINE AND HAS DECREASED ELASTASE INHIBITORY CAPACITY

This study was undertaken to test directly whether methionine sulfoxide residues could be demonstrated in α_1-proteinase inhibitor (α_1-PI) recovered from the lungs of cigarette smokers. To do this, the elastase inhibitory capacity per mg of α_1-PI was measured in the bronchoalveolar lavage (BAL) fluid from 26 healthy smokers and 24 nonsmokers. Activity was decreased by 40% in smokers' BAL fluid compared to nonsmokers. This effect was demonstrable when either human neutrophil elastase or porcine pancreatic elastase was used as test enzyme and was reproducible when selected individuals in each group underwent lavage on repeated occasions. In contrast, the functional activity of α_1-antichymotrypsin was not decreased in smokers' BAL fluid. Crossed antigen-antibody electrophoresis confirmed that inactivation of α_1-PI was responsible for the decrease in the elastase inhibitory capacity of smokers' BAL fluid. Specifically, α_1-PI purified from smokers' BAL fluids contained methionine sulfoxide, whereas α_1-PI from nonsmokers' BAL fluid did not. Smokers' α_1-PI was indistinguishable from nonsmokers' α_1-PI on the basis of electrophoretic mobility, molecular weight, and immunoreactivity. Thus, oxidation of methionine residues in lung α_1-PI is associated with cigarette smoking. Because chemical oxidation of α_1-PI *in vitro* causes loss of its elastase inhibitory activity, the observations presented here suggest that methionine oxidation may also be related to decreased functional activity of lung α_1-PI in smokers *in vivo*.

Carp, H., Miller, F., Hoidal, J. R., and *Janoff, A.*

Proceedings of the National Academy of Sciences of the United States of America 79(6):2041-2045, 1982.

Other support: U.S. Public Health Service.

From the Department of Pathology, State University of New York at Stony Brook, Stony Brook, and the Department of Internal Medicine, University of Minnesota Health Sciences Center, Minneapolis.

OXIDATION OF ALPHA$_1$-PROTEINASE INHIBITOR AS A MAJOR, CONTRIBUTING FACTOR IN THE DEVELOPMENT OF PULMONARY EMPHYSEMA

The importance of oxidation of alpha$_1$-proteinase inhibitor (α_1-PI) in the development of pulmonary emphysema is considered carefully in this paper. Earlier work has shown that neutrophil elastase and cathepsin-G are responsible for elastin degradation in the lung and that the major function of α_1-PI is to control elastolytic activity. Other work has shown that the reactive site of α_1-PI contains a methionyl-seryl peptide bond. Treatment with SucNC1 chemically oxidizes two of the methionyl residues in the inhibitor to the sulfoxide form, one of which is the reactive site methionine. Two methionyl residues of α_1-PI (and no other amino acids) are also oxidized by the action of the neutrophil enzyme, myeloperoxidase. On NaDodSO$_4$-acrylamide gel electrophoresis, myeloperoxidase-treated α_1-PI has the same molecular weight as native α_1-PI, but cannot form a complex with porcine elastase. In fact, the molecular weight of oxidized α_1-PI is reduced in the presence of elastase, indicating modification of the inhibitor. Sequence analysis demonstrated that proteolytic cleavage occurred at the P_8 methionyl bond and that both the P_8 and P_1 methionyl residues were oxidized. Also, in an earlier environmental experiment, filtered cigarette smoke, bubbled through plasma, decreased elastase inhibitor activity by 3% and unfiltered smoke inhibited the activity by 19%. It is known, however, that the lungs have a vast surface area, which may render inhibitors on the lining more susceptible to oxidants. In the data presented in the present paper, an alternative process for the development of a proteinase-proteinase inhibitor imbalance in tissues and, in particular, in the lung is offered: It is known that many kinds of oxidants are produced by neutrophils and macrophages. Since α_1-PI contains two oxidizable methionyl residues, one essential for activity, an imbalance between proteinases and proteinase inhibitors could readily occur. Eventually, such an imbalance could give rise to emphysema, even in individuals with normal levels of α_1-PI.

Travis, J. et al.

Bulletin européen de Physiopathologie Respiratoire 16 (suppl.):341-351, 1980.

Other support: National Institutes of Health.

From the Department of Biochemistry, University of Georgia, Athens.

DETERMINATION OF OXIDIZED ALPHA-1-PROTEINASE INHIBITOR IN SERUM

This methodology paper describes an assay for oxidized alpha-1-proteinase inhibitor (α_1-PI) based on a measurable difference between the inhibitory activities of normal and oxidized α_1-PI against trypsin and elastase. Normal α_1-PI inhibits both porcine trypsin and porcine pancreatic elastase. Oxidized α_1-PI has lost its inhibitory activity toward porcine pancreatic elastase while retaining its net porcine trypsin inhibitory capacity, although the rate of association with trypsin is markedly reduced. On noting this difference, a rapid procedure was developed for determining the percentage of oxidized α_1-PI in plasma or serum based on the ratio of trypsin inhibitory

capacity (TIC) to elastase inhibitory capactity (EIC) of α_1-PI in these tissues. The TIC/EIC ratio is not influenced by the other proteins in serum nor by the concentration of α_1-PI. When this technique was adapted to measure the proportion of oxidized α_1-PI in the serum of young adult, healthy smokers and nonsmokers, 23% oxidized inhibitor was found in the smokers' sera, whereas no oxidized α_1-PI was detectable in sera of nonsmokers. Thus smoking appeared to lead to oxidation damage to circulating α_1-PI. Reduction of the EIC of the α_1-PI in the serum of smokers was compensated by a 1.43-fold increase in their serum α_1-PI titers. This assay for oxidized α_1-PI may be useful in studies of the relationship between oxidation of α_1-PI and the development of pulmonary emphysema.

Beatty, K., Robertie, P., Senior, R. M., and *Travis, J.*

The Journal of Laboratory and Clinical Medicine 100(2):186-192, 1982.

Other support: National Institutes of Health.

From the Department of Biochemistry, University of Georgia, Athens, and The Department of Medicine, Washington University School of Medicine at The Jewish Hospital of St. Louis, St. Louis.

ALPHA$_1$-PROTEINASE INHIBITOR IS MORE SENSITIVE TO INACTIVATION BY CIGARETTE SMOKE THAN IS LEUKOCYTE ELASTASE

In these comparative studies, aqueous solutions of gas phase cigarette smoke were incubated with pure human leukocyte elastase or with crude human leukocyte granule extract, and the effects on enzyme activity were determined using a synthetic amide substrate. Simultaneously, the same smoke solutions were incubated with 10% human serum under identical conditions, and the effects on serum inhibition of purified or crude leukocyte elastase were similarly measured. The *in vitro* results showed that serum elastase inhibiting capacity (α_1-PI) is more susceptible to inactivation by cigarette smoke than is granulocyte elastase, when these proteins are incubated with water-soluble smoke extracts for relatively short times. Similar results were obtained with both synthetic (amide) and natural (elastin) substrates, gas phase smoke and unfractionated whole smoke, and with pure enzyme and crude leukocyte granule extract. Also, aqueous solutions of unfractionated cigarette smoke were incubated with leukocyte elastase or serum, and the abilities of the smoke-treated enzyme to digest elastin and of the smoke-treated serum to inhibit elastin digestion were determined. Both experimental protocols showed that serum elastase-inhibiting capacity (primarily caused by α_1-PI) is more susceptible to inactivation by aqueous solutions of cigarette smoke than is leukocyte elastase, suggsting that elastase inhibition (rather than elastase activity) may be predominantly suppressed by cigarette smoke inhalation *in vivo*.

Janoff, A. and Dearing, R.

American Review of Respiratory Disease 126(4):691-694, 1982.

Other support: U. S. Public Health Service.

From the Department of Pathology, State University of New York at Stony Brook, Stony Brook.

ENZYMATIC OXIDATION OF ALPHA-1-PROTEINASE INHIBITOR IN ABNORMAL TISSUE TURNOVER

This review paper describes present knowledge with regard to the interaction of alpha-1-proteinase inhibitor (α_1-PI) and myeloperoxidase *in vitro*, using both purified enzyme and polymorphonuclear leukocytes as study souces. In the first place, α_1-PI was found to contain a methionyl residue at its reactive center which was sensitive to oxidation by either N-chlorosuccinimide or by enzymatic oxidation. Chemical oxidation revealed that two of the eight methionyl residues present in the molecule were oxidized, one of which was at the reactive center. Subsequently, it was found that the enzymatic oxidation by myeloperoxidase from neutrophil granules, in the presence of hydrogen peroxide and halide ion, could also be easily attained. Since myeloperoxidase is present in the same granules which contain proteolytic enzymes, it would be expected that both types of enzymes would be released simultaneously during either phagocytosis or cell turnover. It is worth noting that the myeloperoxidase system does not oxidize or inactivate most other proteinase inhibitors or proteinases. In the second place, it was shown recently that when α_1-PI was exposed to viable neutrophils in the presence of chloride ion and phorbol myristyl acetate, the abiltiy of α_1-PI to bind elastase was diminished. However, when myeloperoxidase-deficient cells or those from patients with chronic granulomatous disease were used, there was no effect on α_1-PI activity, indicating the involvement of myeloperoxidase and hydrogen peroxide in α_1-PI inactivation. More recently, oxidized α_1-PI has been isolated from rheumatoid synovial fluid of arthritic patients and also from lung lavage fluids of smokers. Approximately four methionyl residues were found to be oxidized in either case, compared to two oxidized residues when native α_1-PI was treated with either chemical or enzymatically produced oxidants. The indication is, therefore, that oxidative inactivation of α_1-PI does occur *in vivo*. Other studies discussed here deal with the importance of oxidation to emphysema development, the actions of the myeloperoxidase system, and the possible role of macrophages in the oxidation of α_1-PI.

Matheson, N. R., Janoff, A. and *Travis, J.*

Molecular and Cellular Biochemistry 45:65-71, 1982.

From the Department of Biochemistry, University of Georgia, Athens, and the Department of Pathology, State University of New York at Stony Brook, Stony Brook.

RAPID CONVERSION OF ANGIOTENSIN I TO ANGIOTENSIN II BY NEUTROPHIL AND MAST CELL PROTEINASES

Angiotensin II is a peptide that has potent vasoconstrictor and aldosterone secretion activities and is derived from the larger parent protein angiotensinogen, which circulates in the plasma. On the other hand, angiotensin I is a peptide that has no significant biological activity and requires further conversion to angiotensin II. In the studies reported here, it is shown that human neutrophil cathepsin G and human skin mast cell chymase rapidly convert angiotensin I to angiotensin II with only minor cleavage elsewhere in the molecule. The rate of cleavage is consistent with a potential role for either or both of these enzymes in an alternate pathway for angiotensin II

synthesis. Since neither enzyme is inhibited by captopril, an angiotensin converting enzyme inactivator, it is possible that leukocyte and mast cell enzymes may play a significant role in the development of abnormally high local concentrations of angiotensin II, associated with various inflammatory processes.

Reilly, C. F., Tewksbury, D. A., Schechter, N. M., and *Travis, J.*

The Journal of Biological Chemistry 257(15):8619-8622, 1982.

Other support: National Institutes of Health.

From the Department of Biochemistry, University of Georgia, Athens, the Marshfield Foundation for Medical Research, Marshfield, WI, and the Department of Medicine, Duke University School of Medicine, Durham, NC.

THE EFFECT OF α_2-MACROGLOBULIN ON THE INTERACTION OF α_1-PROTEINASE INHIBITOR WITH PORCINE TRYPSIN

The dissociation of α_1-proteinase inhibitor:porcine trypsin complex is rapid and has permitted the experiments reported here. In this study, the rate of dissociation of the α_1-proteinase inhibitor:porcine trypsin complex by itself was compared with the rate of dissociation of the same complex in the presence of α_2-macroglobulin. In the presence of the latter inhibitor the dissociation was more rapid, and active α_1-proteinase inhibitor could be recovered in the mixture. However, no active inhibitor could be detected after dissociation in the absence of α_2-macroglobulin. This recovery of active α_1-proteinase inhibitor from complexes with porcine trypsin is the first demonstration of a thermodynamic equilibrium between this inhibitor and proteinase. Consequently, the transfer of trypsin from complexes with α_1-proteinase inhibitor to α_2-macroglobulin may be explained as a passive phenomenon which does not require a physical collision between α_2-macroglobulin and the α_1-proteinase inhibitor:porcine trypsin complex. The dissociation of the complex occurs more rapidly in the presence of α_2-macroglobulin because this inhibitor complexes trypsin leaving the α_1-proteinase inhibitor:porcine trypsin complex by both the irreversible breakdown step and by reversible dissociation of the complex.

Beatty, K., *Travis, J.* and Beith, J.

Biochimica et Biophysica Acta 704:221-226, 1982.

Other support: National Institutes of Health.

From the Department of Biochemistry, University of Georgia, Athens, and the Faculté de Pharmacie, Laboratoire d'Enzymologie, Université Louis Pasteur, Strasbourg, France.

ENZYMATIC REDUCTION OF OXIDIZED α-1-PROTEINASE INHIBITOR RESTORES BIOLOGICAL ACTIVITY

Oxidation easily inactivates α_1-proteinase inhibitor (α_1-PI), the major serum inhibitor of proteolytic activity. The noted inactivation seems to be due to the oxida-

tion of an essential methionine residue(s) in α_1-PI that is required for the inhibition of elastase activity. When methionine residue(s) in α_1-PI are oxidized, they result in Met(0). In theory, the presence of an enzyme in cells that can reduce Met(0) residues in proteins to methionine provides a mechanism for restoring biological activity to a protein that has been inactivated because of oxidation of an essential methionine residue. It has been shown elsewhere that extracts of *Escherichia coli* contain an enzyme that can reduce Met(0) residues in protein to methionine. This study shows that oxidized, functionally inactive canine α_1-PI does indeed regain its biological inhibitory activity after reduction with a partially purified preparation of *E. coli* Met(0) peptide reductase.

Abrams, W. R. *Weinbaum, G.*, Weissbach, L., Weissbach, H., and Brot, N.

Proceedings of the National Academy of Sciences of the United States of America 78(12):7483-7486, 1981.

Other support: National Heart, Lung, and Blood Institute.

From the Department of Medicine, Albert Einstein Medical Center, Philadelphia; Department of Biochemistry and Molecular Biology, University of Florida, Gainesville; and Roche Institute of Molecular Biology, Nutley, NJ.

NEUTROPHIL DEGRANULATION IN CADMIUM CHLORIDE-INDUCED ACUTE LUNG INFLAMMATION

Cadmium chloride ($CdC1_2$), a toxic chemical that has been reported to cause centrilobular or scar emphysema, was used in this study to induce neutrophil migration into the alveolar spaces as a model of lung inflammation. Results showed that lobar intrabronchial instillation of $CdC1_2$ (200 μg/ml) in saline causes a reproducible acute pulmonary inflammation in dogs. The influx of inflammatory neutrophils from the circulation into the alveolar spaces reaches a maximum approximately 16 hours after the $CdC1_2$ treatment in the treated lobe, while the contralateral lung appears normal. Morphometric quantitation of peroxidase-positive (azurophilic) granules in the inflammatory neutrophils shows a 74% loss of these granules, with little or no loss of the peroxidase-negative (specific) granules. These data are in good agreement with the measured loss of intracellular elastase, an enzyme known to be localized in the azurophilic granules. The results suggest that degranulation of azurophilic granules may occur selectively during this chemically-induced acute inflammation.

Yamada, H., Damiano, V. V., Tsang, A-L., Meranze, D. R., Glasgow, J., Abrams, W. R., and *Weinbaum, G.*

American Journal of Pathology 109:145-156, 1982.

Other support: National Heart, Lung, and Blood Institute.

From the Research Division, Department of Medicine, Albert Einstein Medical Center, Philadelphia, and the Franklin Research Center, Philadelphia.

PARASYMPATHETIC INNERVATION OF THE CERVICAL TRACHEALIS MUSCLE IN LIVING DOGS

Use of the trachea to study airway function in living dogs permits examination of a prototypic central airway under relatively convenient circumstances using established

technology. In the study presented here, the pathways by which parasympathetic fibers were carried to the cervical trachaelis muscle were characterized in 34 anesthetized dogs. Nerves were stimulated electrically, and tracheal tension was monitored in segments of the posterior membrane *in vivo*. Stimulation of superior laryngeal nerves contracted 34 of 34 cranial cervical segments and two of four caudal cervical segments. Recurrent laryngeal nerves contributed to innervation of 34 of 34 cranial, as well as four of four caudal, segments. Stimulation of paracurrent nerves contracted 11 of 34 cranial and four of four caudal segments. Mechanical effects of esophageal contraction, induced by stimulating pararecurrent nerves, did not alter tension in tracheal segments. Tracheal contractions induced by stimulation of all three pathways simultaneously were significantly less than the sum of contractions produced by stimulating each set individually; this was probably due to anastomoses between terminal neurons, overlap in their anatomic distribution, or intercellular nexuses in trachealitis muscle. It seems, therefore, that parasympathetic innervation of the canine trachea is by three different neuroanatomic pathways.

Brown, J. K., Shields, R. and *Gold, W. M.*

Journal of Applied Physiology: Respirat, Environ. Exercise Physiol. 53(3):617-625, 1982.

Other support: National Heart, Lung and Blood Institute.

From the Cardiovascular Research Institute and Department of Medicine, University of California, San Francisco.

EFFECT OF SMOKING A CIGARETTE ON THE DENSITY DEPENDENCE OF MAXIMAL EXPIRATORY FLOW

Earlier studies of these investigators have shown that tobacco smoke causes an increase in airways resistance and a drop in expiratory flow at 50% of the vital capacity (FEF_{50}). In the present attempt to gain further insight into the nature and site of this bronchoconstrictive effect, maximal expiratory flow was measured in 12 healthy volunteers while they were breathing air and a low-density gas mixture (helium-oxygen) before and after smoking a cigarette. Results showed that prior to smoking the forced vital capacity (FVC) measured while breathing air was not significantly different from that obtained while breathing the helium-oxygen mixture. Smoking did not cause any changes in FVC. However, after smoking, the FEF_{50} measured while breathing air decreased significantly, while smoking did not cause any changes in the FEF_{50} obtained after breathing the low-density gas mixture. $\Delta\dot{V}max_{50}$ increased from 47.1± 11.4% before smoking to 57.0 ± 13.3% after smoking. There were no changes in flow at 75% FVC and volume of isoflow. These observations are discussed in light of the equal pressure point (EPP) analysis and wave speed theory of flow limitation. It was concluded that after smoking, flow becomes more density dependent because there is constriction of a flow-limiting segment downstream from the EPP, located in lobar and segmental bronchi. No acute effect of tobacco smoke on the small airways could be demonstrated.

Taveira Da Silva, A. M. and *Hamosh, P.*

Respiration 43:258-262, 1982.

From the Departments of Medicine, Physiology and Biophysics, Georgetown University Schools of Medicine and Dentistry, Washington, DC.

RESPIRATORY AND CARDIOVASCULAR EFFECTS OF INTRAVENTRICULAR CHOLECYSTOKININ

In this attempt to assess the role of cholecystokinin- and gastrin-like peptides in the central, regulatory control of respiratory and cardiovascular functions, cholecystokinin in doses of 1-300 ng was administered into the lateral brain ventricle of chloralose-anesthetized cats while tracheal airflow, arterial blood pressure, and heart rate were monitored. Results showed that the most striking effect was an increase in respiratory activity. This was observed with a dose as low as 1 ng and peaked with a dose of 100 ng. Respiratory stimulation was indicated by an increase in respiratory minute volume, an increase that was due to an increase in tidal volume as no significant effect was noted on respiratory rate, inspiratory time, expiratory time, and total respiratory cycle duration. On the other hand, when 300-1000 ng of cholecystokinin were administered intravenously, no respiratory stimulant effect was observed. These results indicate that cholecystokinin acts in the brain to stimulate respiration.

Pagani, F. D., Taveira Da Silva, A. M., *Hamosh, P.*, Garvey, T. Q., III and Gillis, R. A.

European Journal of Pharmacology 78:129-132, 1982.

Other support: American Heart Association.

From the Departments of Pharmacology, Physiology and Medicine, Georgetown University Schools of Medicine and Dentistry, Washington, DC.

CIGARETTE SMOKE CONTAINS ANTICOAGULANTS AGAINST FIBRIN AGGREGATION AND FACTOR XIIIa IN PLASMA

In this *in vitro* study, the effect of cigarette smoke on fibrin aggregation was investigated by the use of water-soluble, gas-phase components of smoke, obtained by bubbling the smoke produced from one cigarette through three ml of buffer or distilled water. This extract was incorporated in varying dilutions in the buffer to which fibrin monomer solution was added in order to initiate fibrin aggregation. Results showed a dose-dependent delay in fibrin aggregation. Increasing the amount of smoke extract resulted in decreased absorbance of the clot and delayed onset of fibrin aggregation. The fibrin aggregation inhibitor was also examined by use of two differing fibrin preparations with α chains lacking COOH-terminal segments. From the sum of these studies, it was seen that cigarette smoke contains two distinct coagulation inhibitors: one which prolongs the clotting times of plasma by inducing delayed fibrin aggregation and requiring the COOH-terminal region of fibrin α chains to exert its effect; the other inactivates XIIIa, thus preventing the cross-linking of fibrin polymers. These anticoagulant properties of smoke are demonstrable in plasma, where they may play a role in the physiology of smoking.

Galanakis, D. K., Laurent, P., *Janoff, A.*, and Chung, S. I.

Science 217:642-645, 1982.

Other support: U. S. Public Health Service.

From the Health Science Center, State University of New York at Stony Brook, Stony Brook, and the National Institute of Dental Research, Bethesda, MD.

PLATELETS INCREASE NEUTROPHIL ADHERENCE *IN VITRO* TO NYLON FIBER

Interaction of neutrophils with platelets is a common phenomenon that may be important in the pathogenesis of various diseases. Although platelets are known to have a strong affinity for foreign surfaces, their effect on the measured adherence of neutrophils in the commonly used nylon fiber systems had not been determined. Therefore, in the study reported here, the effect of platelets on the adherence of neutrophils to nylon fiber was assessed in whole blood samples and purified neutrophil suspensions in the presence or absence of plasma. Measurements showed that in whole blood samples, increasing numbers of platelets were associated with increasing adherence of neutrophils. Addition of platelets in plasma to purified neutrophil suspensions increased ($p<0.05$) neutrophil adherence from 76.2% ± 1.4 to 88.0% ± 2.0. Similarly, addition of washed platelets without plasma also increased ($p<0.05$) neutrophil adherence from 67.9% ± 5.8 (without added platelets) to 94.2% ± 1.6 (with 300,000 platelets/mm³ added). In contrast, no augmentation of neutrophil adherence occurred if platelets had their aggregation response suppressed by pretreating platelet donors with aspirin. Scanning electron microscopy supported these findings by showing platelets in close association with neutrophils adhering to nylon fiber. These findings emphasize the importance of platelet numbers and reactivity on the adherence of neutrophils.

Rasp, F. L., Clawson, C. C. and *Repine, J. E.*

The Journal of Laboratory and Clinical Medicine 97(6):812-819, 1981.

Other support: American Lung Association, Minnesota Medical Foundation, National Institutes of Health, American Heart Association, Kroc Foundation, and the Graduate School of the University of Minnesota.

From the Departments of Medicine and Pediatrics, University of Minnesota Health Sciences Center, Minneapolis, and the Webb-Waring Lung Institute and Department of Internal Medicine (Pulmonary), University of Colorado Health Sciences Center, Denver.

A NOVEL MECHANISM FOR PULMONARY OXYGEN TOXICITY: PHAGOCYTE MEDIATED LUNG INJURY

In initial studies, a significant increase in the number of polymorphonuclear leukocytes (PMN) in the alveolar lavages of rats exposed to hyperoxia for three days was noted. This observation led to two hypotheses. The first hypothesis, that hyperoxia causes injury to alveolar macrophages (AM), inducing their release of chemotactic factors which recruit PMNs to the lung, was supported by evidence that: (1) hyperoxia damages AM *in vivo* and *in vitro*, and (2) chemotactic factors from alveolar lavages of hyperoxia-exposed animals are biochemically similar to those released by AM exposed to hyperoxia in cell culture. The second hypothesis, that PMNs play an important role in pulmonary oxygen toxicity, was supported by the finding that the degree of endothelial damage was highly correlated with the numbers of PMNs recovered in alveolar lavages in both neutrophil-sufficient and neutropenic rabbits. However, two major

unknowns still remain. First, how does hyperoxia damage AM? Second, how do PMNs damage endothelium? At this time, a reasonable working hypothesis is that the damage in each case is due to the formation of oxygen radicals, particularly the highly reactive hydroxyl radicals. Improved understanding of the role of AM, PMN and hydroxyl radical in lung damage due to hyperoxia will be important in elucidating basic mechanisms involved in the pathogenesis of pulmonary oxygen toxicity, as well as in other types of environmental lung injury.

Fox, R. B., Shasby, D. M., Harada, R. N., and *Repine, J. E.*

CHEST 80S:3S-4S, 1981.

Other support: American Heart Association, National Institutes of Health, and the Maytag-Crawford Trust Fund.

From the Experimental Medicine Division, Webb-Waring Lung Institute, University of Colorado Health Sciences Center, Denver.

POTENTIAL MECHANISMS OF LUNG INJURY FROM HYDROXYL RADICAL

Earlier biochemical studies have indicated that inhaled environmental toxins may, in one way or another, contribute to lung damage. For example, there is evidence that polymorphonuclear leukocytes (PMNs) are efficient at making highly reactive O_2^- intermediates and since factors released from alveolar macrophages (AM) exposed to hyperoxia can stimulate release of O_2^- intermediates from PMN, it appears likely that O_2^- intermediates are involved in this injury. More recent work has focused on the killing of bacteria by PMN as a model of PMN-induced lung damage. In one of these studies, increasing concentrations of DMSO or thiourea progressively and significantly decreased killing of *Staphylococcus aureus*, 502A, by normal PMN but did not further decrease the abnormal bactericidal activity of CGD PMN which fail to produce •OH. For studies with Fe^{++} it was hypothesized that *S. aureus* was providing a majority of the substances(s), such as Fe^{++}, which then reacted with H_2O_2 to generate •OH and CH_4 from DMSO. The results of these studies indicate that hydroxyl radical (•OH) plays an important role in the killing of *S. aureus* by human PMN. Other studies suggested that •OH might also be formed and participate in the killing of *S. aureus* in another way. Elucidation of this latter mechanism was an outgrowth of studies done to determine the bactericidal activity of chemical system, Fe^{++} and H_2O_2, which generates •OH. In these studies it appears that H_2O_2 reacts with Fe^{++} in the bacteria to produce •OH by a Fenton type reaction. Hydroxyl radical then injures *S. aureus* by reacting with key organic molecules. It was also noted here that DMSO prevents injury to *S. aureus* by scavenging and detoxifying •OH.

Repine, J. E. et al.

CHEST 80S:45S-48S, 1981.

Other support: American Heart Association, National Institutes of Health, and the Maytag-Crawford Trust Fund.

From the Webb-Waring Lung Institute and the Division of Pulmonary Medicine, University of Colorado Health Sciences Center, Denver.

HYDROGEN PEROXIDE KILLS *STAPHYLOCOCCUS AUREUS* BY REACTING WITH STAPHYLOCOCCAL IRON TO FORM HYDROXYL RADICAL

In the investigation reported here, an attempt was made to elucidate the mechanism underlying the biochemical role of iron in bacterial host-defense interactions. Results showed that two different lines of investigation supported the premise that killing of *Staphylococcus aureus*, 502A, by hydrogen peroxide involves formation of the more toxic hydroxyl radical (•OH) through the iron-dependent Fenton reaction. First, growing *S. aureus* overnight in broth media with increasing concentrations of iron increased their content of iron and dramatically enhanced their subsequent susceptibility to killing by H_2O_2. Second, in direct relation to their effectiveness as •OH scavengers, thiourea, dimethyl thiourea, sodium benzoate, and dimethyl sulfoxide inhibited H_2O_2-mediated killing of *S. aureus*. Therefore, it seems from this work that, while iron is an essential growth nutrient, it may also provide an "Achilles heel" for *S. aureus*, indicating that the reported mechanism may be a new way in which iron is beneficial to the host.

Repine, J. E. Fox, R. B. and Berger, E. M.

The Journal of Biological Chemistry 256(14):7094-7096, 1981.

Other support: National Institutes of Health, American Heart Association, and the Kroc Foundation.

From the Webb-Waring Lung Institute and the Pulmonary Divisions of the Departments of Medicine and Pediatrics, University of Colorado Health Sciences Center, Denver.

SERUM FROM PATIENTS WITH INVASIVE FUNGAL INFECTIONS INHIBITS THE ADHERENCE OF POLYMORPHONUCLEAR LEUKOCYTES AND ALVEOLAR MACROPHAGES

Although considerable attention has been directed recently toward determining the effect of bacterial infection on neutrophil adherence, little attention has been paid so far to the influence of fungal infection on phagocyte adherence. In the investigation reported here, however, the adherence of various combinations of polymorphonuclear leukocytes (PMN) or alveolar macrophages (AM) and serum from patients with fungal infections or from control subjects was evaluated *in vitro* using the standard nylon fiber pipette technique. Results showed that the intrinsic adherence of AM and PMN from patients with a wide variety of untreated fungal infections was normal, but that these patients had a serum inhibitor that could reversibly decrease the adherence of PMN and AM. Specifically, studies using various combinations of PMN and serums from patients with blastomycosis suggested that the adherence defect was due to a serum disorder rather that an intrinsic cellular abnormality. While preincubation in serum from patients with blastomycosis decreased the adherence of control PMN, preincubation in control serum corrected the decreased adherence of PMN from patients with blastomycosis. On the basis of these and other related studies, it was concluded that the intrinsic adherence of PMN and AM from patients with untreated fungal infections is normal, but that these patients have an extrinsic heat-labile serum factor that can decrease the adherence of PMN and AM.

Rasp, F. L., Sarosi, G. A. and *Repine, J. E.*

American Review of Respiratory Disease 123:636-639, 1981.

Other support: Minnesota Medical Foundation, Minnesota and American Lung Associations, National Institutes of Health, American Heart Association, and the Kroc Foundation.

From the Departments of Medicine of the University of Minnesota Health Sciences Center and the Minneapolis Veterans Administration Medical Center, and the Webb-Waring Lung Institute and the Department of Medicine of the University of Colorado Health Sciences Center, Denver.

ANGIOTENSIN CONVERTING ENZYME CONCENTRATIONS IN THE LUNG LAVAGE OF NORMAL RABBITS AND RABBITS TREATED WITH NITROGEN MUSTARD EXPOSED TO HYPEROXIA

Granulocytes have been implicated recently in the development of acute hyperoxic lung injury, an edematous lung mishap that is characterized grossly by massive edema and histologically by endothelial injury with perivascular inflammation. In the present study, specifically, increased concentrations of angiotensin converting enzyme (ACE) were found in lung lavages from rabbits exposed for 72 hours to hyperoxia and the concentrations of ACE were correlated with ratios of extravascular lung water to body weight and albumin concentrations in lung lavages. In parallel studies, rabbits treated with nitrogen mustard in which granulocytopenia was maintained throughout the 72-hour hyperoxic exposure period had less evidence of edematous lung injury and lower concentrations of ACE in their lung lavages than similarly treated rabbits in which granulocytopenia was not maintained. These results suggested that granulocytes contribute to acute edematous lung injury from hyperoxia and that ACE concentrations in lung lavages reflect this process.

Shasby, D. M., Shasby, S. S., Bowman, C. M., Fox, R. B., Harada, R. M., Tate, R. M., and *Repine, J. E.*

American Review of Respiratory Disease 124:202-203, 1981.

Other support: National Heart, Lung and Blood Institute, American Heart Association, National Institutes of Health, and the Kroc Foundation.

From the Webb-Waring Lung Institute, Department of Medicine (Pulmonary Sciences), University of Colorado Health Sciences Center, Denver.

NEUTROPHILS AND LUNG EDEMA: STATE OF THE ART

This brief review focuses on the emerging role of neutrophils and their products in the development of adult respiratory distress syndrome (ARDS), the most common presenting form of edematous lung injury. For some time, the association of ARDS with multiple inciting events has led to the presumption that, under certain circumstances, many factors might be involved in the pathogenesis of ARDS. While not firmly established, a loss of alveolar-capillary membrane integrity which results in lung edema appears to be a pathologic change common to all cases of ARDS, thus

making it possible for cellular or humoral components to contribute to endothelial injury. A number of recent observations suggest that neutrophils contribute to the development of edematous lung injury. First, increased numbers of neutrophils are commonly found in lung lavages of patients with ARDS and early in their illness. Second, neutrophil depletion protects animals from experimental edematous lung injury, and third, neutrophils have been shown to have potent mechanisms for causing tissue injury, altering vascular permeability and perturbing hemodynamics. Overall, considerable evidence is now available to suggest that neutrophils participate in the development of acute edematous lung injury such as that seen in patients with ARDS. The mechanisms by which neutrophils might mediate these effects appear to be many, varied and still ill-defined at this time.

Repine, J. E., Bowman, C. M., and Tate, R. M.

CHEST 81S:47S-50S, 1982.

Other support: National Institutes of Health, American Heart Association and the Kroc Foundation.

From the Webb-Waring Lung Institute, University of Colorado Health Sciences Center, Denver.

ALVEOLAR MACROPHAGE SECRETIONS: INITIATORS OF INFLAMMATION IN PULMONARY OXYGEN TOXICITY?

In the paper presented here, attempts were made to elucidate mechanisms responsible for neutrophil influx into the lungs and to understand more clearly the contribution of neutrophils to the undefined pathogenesis of pulmonary oxygen toxicity. For this study, alveolar macrophages (AM), following lavage from lungs of infection-free New Zealand White rabbits, were exposed in culture to normoxia (15% O_2) or hyperoxia (95% O_2) for periods up to 72 hrs. Supernatants from AM cultures were evaluated for their chemotactic, adherence stimulating, and superoxide radical stimulating activities for neutrophils. Results showed that hyperoxia damages and stimulates alveolar macrophages to release factors which affect neutrophil recruitment, adherence and activation. Preliminary characterization of these factors suggests that the factors have separate identities. Factors derived from AM exposed to hyperoxia differ in molecular weight, heat stability, and time of maximal activity. The stimulus for macrophage release of these factors may involve damage to AM by hyperoxia. These observations support the possibility that AM and the proposed mechanism of neutrophil recruitment and activation may be important in the pathogenesis of oxygen toxicity and other forms of acute lung injury.

Harada, R. N., Bowman, C. M., Fox, R. B., and *Repine, J. E.*

CHEST 81S:52S-54S, 1982.

Other support: American Lung Association of Colorado, American Heart Association, National Institutes of Health, the Kroc Foundation, Hill Foundation, Swan Foundation, and Kleberg Foundation.

From the Webb-Waring Lung Institute, Pulmonary Divisions, University of Colorado Health Sciences Center, Denver.

OXYGEN RADICAL-INDUCED PULMONARY EDEMA: A MECHANISM FOR THE PRODUCTION OF NONCARDIOGENIC PULMONARY EDEMA BY NEUTROPHILS

Since stimulated neutrophils make O_2 radicals and since O_2 radicals have been shown to damage a number of biological tissues, it seemed possible that release of O_2 radicals from stimulated neutrophils might perturb the alveolar-capillary membrane and lead to protein-rich edema formation. To test this hypothesis, isolated perfused lungs from New Zealand White rabbits were monitored and, after a stable baseline period, purine and xanthine oxidase were introduced into the perfusate with or without prior introduction of O_2 radical scavengers. Results of this investigation showed that the chemical generation of O_2 radicals by intravascular injection of purine and xanthine oxidase resulted in acute protein-rich edema formation in the isolated lungs. This edematous process was markedly inhibited by the prior individual injection of several O_2 radical scavengers, including catalase, dimethyl sulfoxide, and dimethylthiourea. These findings provide direct evidence to suggest that O_2 radicals are capable of perturbing the air-blood barrier and causing a protein-rich edema. They also suggest that the intra-vascular release of O_2 radicals from stimulated neutrophils might be capable of producing a similar acute edematous lung injury in certain clinical settings.

Tate, R. M., Shasby, D. M., VanBenthuysen, K. M., McMurthy, I. F., and *Repine, J. E.*

CHEST 81S:57S-59S, 1982.

Other support: American Lung Association of Colorado, American Heart Association, National Institutes of Health, the Kroc Foundation, Hill Foundation, Swan Foundation, and the Kleberg Foundation.

From the Webb-Waring Lung Institute, Pulmonary Division, Cardiovascular Pulmonary Research Laboratory and the Departments of Medicine and Pediatrics, University of Colorado Health Sciences Center, Denver.

REDUCTION OF THE EDEMA OF ACUTE HYPEROXIC LUNG INJURY BY GRANULOCYTE DEPLETION

The relationship between granulocytes in the lung and the edema of acute hyperoxic lung injury was examined in the study reported here. Results showed that although increased numbers of granulocytes are found in lungs acutely injured by hyperoxia, their contribution to lung injury remains unknown. It was found that circulating granulocytes increased markedly in rabbits exposed to hyperoxia for 72 hrs. and that the number of granulocytes in lung lavages also increased and were correlated with the degree of edematous lung injury. Furthermore, when rabbits were treated with nitrogen mustard (1.75 mg/kg) and developed sustained granulocytopenia, exposure to hypertoxia for 72 hrs. resulted in fewer granulocytes in lung lavages and less edematous lung injury. In contrast, when rabbits were similarly treated with nitrogen mustard but did not maintain sustained granulocytopenia throughout the exposure to hyperoxia, increased numbers of granulocytes were found in lung lavages and the degree of

edematous lung injury increased to levels not different from those observed in oxygen-exposed rabbits that had not been treated with nitrogen mustard. These findings suggest that granulocytes may contribute to production of edema in acute oxygen toxicity.

Shasby, D. M., Fox, R. B., Harada, R. N. and *Repine, J. E.*

Journal of Applied Physiology: Respirat. Environ. Exercise Physiol. 52 (5): 1237-1244, 1982.

Other support: National Heart, Lung and Blood Institute, American Heart Association, and the Kroc Foundation.

From the Webb-Waring Lung Institute, Department of Internal Medicine (Pulmonary Sciences Division), University of Colorado Health Sciences Center, Denver.

GRANULOCYTES MEDIATE ACUTE EDEMATOUS LUNG INJURY IN RABBITS AND IN ISOLATED RABBIT LUNG PERFUSED WITH PHORBOL MYRISTATE ACETATE: ROLE OF OXYGEN RADICALS

Many forms of acute noncardiogenic lung edema are associated with an accumulation of inflammatory cells in the alveoli and microvessels of the lung, and there have been many suggestions that the activated granulocytes play an important role in this process. In the study reported here explicitly, it was seen that intravenously injected phorbol myristate acetate (PMA) caused a protein-rich edema in lungs of control rabbits but not in granulocytopenic rabbits pretreated with nitrogen mustard. Specifically, control rabbits treated with PMA had higher lung weight to body weight ratios and lung lavage albumin concentrations than granulocytopenic rabbits pretreated with nitrogen mustard and then given PMA. To further clarify the role of granulocytes in the production of edema, additional experiments were conducted in an isolated perfused rabbit lung. Addition of purified granulocytes and PMA to the balanced salt perfusate caused lung edema, whereas neither granulocytes nor PMA alone caused edema. Also, in order to determine the contribution of oxygen radicals to the pathogenesis of the edema, chronic granulomatous disease granulocytes, which are deficient in oxygen radical production, were added to the isolated lung perfusate. Chronic granulomatous disease granulocytes and PMA did not cause edema in isolated lungs, whereas granulocytes from normal human subjects and PMA did. These data suggest that oxygen radicals released from stimulated granulocytes contribute to the pathogenesis of acute edematous lung injury.

Shasby, D. M., VanBenthuysen, K. M., Tate, R. M., Shasby, S. S., McMurtry, I., and *Repine, J. E.*

American Review of Respiratory Disease 125:443-447, 1982.

Other support: American Heart Association, National Institutes of Health, and the Kroc Foundation.

From the Webb-Waring Lung Institute and the Cardiovascular Pulmonary Research Laboratory of the University of Colorado Health Sciences Center, Denver, and the Pulmonary Division of the University of Virginia School of Medicine, Charlottesville.

CYTOCHALASIN B AND THE STRUCTURE OF ACTIN GELS II. FURTHER EVIDENCE FOR THE SPLITTING OF F-ACTIN BY CYTOCHALASIN B

Cytochalasin B, a fungal metabolite that can alter cell shapes and inhibit a wide variety of cellular movements under certain conditions, has been shown in the past to be able to reduce the network structure of actin filaments. In this communication, additional evidence is presented that cytochalasin B shortens actin filaments and that this shortening takes place without net depolymerization. Overall, the work done here showed that cytochalasin B decreased the flow birefringence and $S_{20,w}$ and increased the extinction angle of actin filaments in salt solutions favoring polymerization of the protein. These changes occurred without a detectable increase in the equilibrium actin monomer concentration determined by a radioassay. These results complement earlier observations indicating that cytochalasin B shortens actin filaments without net depolymerization. Analyzed in terms of Flory's classical network theory, this shortening accounts for the marked effect of cytochalasin B in dissolving the gel structure of F-actin crosslinked by actin-binding protein concentration for incipient gelation. Cytochalasin B decreased the annealing rate of low concentrations of actin filament fragments prepared by sonic disruption. This result is consistent with the idea that cytochalasin B binds to the ends of actin filaments, and may explain how cytochalasin B causes filament shortening.

Maruyama, K., Hartwig, J. H. and *Stossel, T. P.*

Biochimica et Biophysica Acta 626:494-500, 1980.

Other support: U. S. Public Health Service.

From the Department of Muscle Research, Boston Biomedical Research Institute; Hematology-Oncology Unit, Massachusetts General Hospital, and the Department of Medicine, Harvard Medical School, Boston.

STRUCTURE OF MACROPHAGE ACTIN-BINDING PROTEIN MOLECULES IN SOLUTION AND INTERACTING WITH ACTIN FILAMENTS

Evidence is presented in this paper that actin-binding protein is a dimer, which has the capacity to initiate and propagate isotropic actin filament networks. For this particular study, the structure of actin-binding molecules was examined in solution and interacting with actin filaments. At physiological ionic strength, actin-binding protein has a M_r value of 540×10^3 as determined by direct and indirect hydrodynamic measurements. It is an assymetrical dimer composed of 270×10^3 dalton subunits. Viewed in the electron microscope after negative staining or low angle shadowing, actin-binding protein molecules assume a broad range of conformations varying from closed circular structures to fully extended strands. All configurations are apparently derived from the same structure which consists of two monomer chains connected end-to-end. Other observations noted in this paper indicate that actin-binding protein dimers are extremely flexible. In further studies, direct visualization of actin-binding protein molecules between actin filaments in the electron microscope showed that dimers are sufficient for crossbridging of actin filaments and that actin-binding protein dimers are bipolar, composed of monomers connected head-to-head and having actin-

binding sites located on the free tails. Overall, it seems apparent from these studies that actin-binding protein is a dimer at physiological ionic strength; each dimer has two actin filament binding sites, and is, therefore, sufficient to get actin filaments in solution. The length and flexibility of the actin-binding protein subunits render this molecule structurally suited for the crosslinking of large helical filaments into isotropic networks.

Hartwig, J. H. and *Stossel, T. P.*

Journal of Molecular Biology 145:563-581, 1981.

Other support: U. S. Public Health Service.

From the Department of Medicine, Massachusetts General Hospital, Boston.

DISTRIBUTION OF ACTIN-BINDING PROTEIN AND MYOSIN IN POLYMORPHONUCLEAR LEUKOCYTES DURING LOCOMOTION AND PHAGOCYTOSIS

There is a theory that actin and proteins that associate with it are the major elements of locomotion and phagocytosis by polymorphonuclear (PMN) leukocytes; and this theory is well supported by the material presented here. In this study, indirect immunoflourescence was used to examine the redistribution of myosin and actin-binding protein (ABP) molecules in rabbit PMN leukocytes during locomotion and phagocytosis. In unpolarized PMN leukocytes, ABP and myosin had a diffuse distribution with some predilection for the cortex. In polarized PMN leukocytes crawling toward yeast particles, myosin and ABP staining concentrated in the anterior pseudopod. In PMN leukocytes fixed during phagocytosis of the yeast particles, antimyosin and anti-ABP staining concentrated strikingly in the distal portions of the pseudopod embracing the yeasts. Staining for catalase, a cytoplasmic protein in PMN leukocytes, for lactoferrin, a protein of specific granules, and for myeloperoxidase, a protein of azyrophilic granules, was not concentrated in pseudopods. Taken together with available morphologic and biochemical information, these findings are consistent with a mechanism wherein interactions of actin, ABP and myosin redistribute cortical cytoplasm into pseudopods involved in locomotion and phagocytosis.

Valerius, N. H., Stendahl, O., Hartwig, J. H. and *Stossel, T. P.*

Cell 24:195-202, 1981.

From the Department of Medicine, Harvard Medical School, and the Hematology-Oncology Unit, Massachusetts General Hospital, Boston.

IDENTIFICATION OF GELSOLIN, A Ca^{2+}-DEPENDENT REGULATORY PROTEIN OF ACTIN GEL-SOL TRANSFORMATION, AND ITS INTRACELLULAR DISTRIBUTION IN A VARIETY OF CELLS AND TISSUES

Gelsolin, a 91,000-dalton globular protein from rabbit lung macrophages, has been shown before to be a major Ca^{2+}-dependent regulatory protein of actin gel-sol

transformation. In the work reported here, antiserum prepared against gelsolin was used to detect the presence of proteins immunologically related to gelsolin in a variety of cells and tissues. Results showed that a single band of cross-reactive material which comigrated with macrophage gelsolin was found in at least nine different kinds of cells and tissues derived from rabbits and humans and in four lines of cultured cells from humans and rats. Gelsolin was also identified in human serum and plasma, raising the possibility that it may contribute to the clearance of actin from the circulatory system. Related studies, using indirect immunoflourescent staining of acetone-fixed macrophages and polymorphonuclear leukocytes, showed that gelsolin resides in the cortical cytoplasm and that during phagocytosis it is concentrated in pseudopodia engulfing particles to be ingested, an area of the cytoplasm actively engaged in movement. In longitudinal cryostat sections of contracted rabbit skeletal muscle, antigelsolin staining was associated with the I-band of the myofibril, suggesting that it may be involved, by an as yet undefined mechanism, in skeletal muscle function. In conclusion, these findings are compatible with the idea that gelsolin is an integral part of the motile apparatus of phagocytic cells and that it regulates cell movement by changing the consistency of the cytoplasm.

Yin, H. A., Albrecht, J. H. and Fattoum, A. (*Stossel, T. P.*)

The Journal of Cell Biology 91:901-906, 1981.

Other support: U. S. Public Health Service and the Edwin S. Webster Foundation.

From the Hematology-Oncology Unit, Massachusetts General Hospital, and the Department of Medicine, Harvard Medical School, Boston.

ACTIN FILAMENTS AND SECRETIONS: THE MACROPHAGE MODEL

In this fact-filled book chapter, the relationship between actin filaments and secretion is scrutinized in several different ways. In one chapter section, morphological and pharmacological evidence is presented for the participation of actin microfilaments in secretion; while, in another section, a macrophage model is employed to characterize the demonstrable association of microfilament rearrangement and secretion, it is noted here that, if the cortical actin microfilament lattice of macrophages is to have some active or passive function in secretion, it must be capable of directional movement. The elements of directional movement are (1) a force-generating mechanism, (2) an orienting influence on the force to provide directionality, and (3) a control mechanism exerted on the force-generating system or on directionality or on both. Each one of these directional movement elements is presented in fuller detail here. In summary, the force-generating mechanism is a superprecipitation of actin and myosin filaments, a process requiring hydrolysis of ATP and presumably based on the sliding-filament interaction chacterized in striated muscle. This energy-dependent mechanism may be a major consumer of the macrophage's metabolic activity and can account for the susceptibility of secretion to inhibition by metabolic poisons. Directionality and amplification of the force generated arise for controlled focal changes in the crosslinking of actin filaments. Gelsolin, a calcium-activated protein, controls lattice rigidity of

actin by severing actin filaments between points of crosslinking by actin-binding protein. The free calcium concentrations that regulate the activity of this protein are levels found in living cells. This mechanism for directional cytoplasmic movement control by calcium is called the "Tug-of-War" hypothesis, and evidence that this mechanism exists in macrophages is presented in this paper.

Stossel, T. P.

Methods in Cell Biology 23:215-229, 1981.

Other support: U.S. Public Health Service, the Edwin S. Webster Foundation and Edwin W. Hiam.

From the Hematology-Oncology Unit, Massachusetts General Hospital, and the Department of Medicine, Harvard Medical School, Boston.

THE MOTOR OF PHAGOCYTIC LEUKOCYTES

There are three things that are needed for cytoplasmic movement: (1) force generation, (2) orientation of the force to provide direction and (3) a control mechanism. The present review summarizes information concerning the biochemical components of the motor of mammalian phagocytes and how they can interact to generate directional movement. In the first place, the motor region of phagocytic leukocytes appears to reside in the peripheral cytoplasm beneath the plasma membrane. Transmission electron micrographs of thin sections of fixed phagocytic leukocytes reveal that this cortical cytoplasm consists primarily of filamentous material, and that the filaments have the dimensions of actin polymers. These actin filaments seem to form a meshwork in which the filaments intersect or overlap at random intervals. Myosin molecules (about 1% of the total protein) of phagocytic leukocytes can form bipolar filaments at physiologic pH and ionic strength, and these myosin filaments bind to actin filaments. Although all of the evidence is not in yet, at this time it seems possible that: (1) the macrophage cortex is an isotropic lattice of actin filaments cross-linked by a protein called actin-binding protein. (2) Myosin filaments, dispersed together with magnesium and ATP throughout the lattice, exert tension on the lattice by means of the crossbridge mechanism, and (3) depending on the calcium concentration, gelsolin, a recently discovered macrophage protein, regulates the structure of the lattice. Comparisons are made in this paper between the motor of mammalian phagocytes and the motor of muscle fibers, and consideration is given to the possible importance of this motor mechanism in cancer biology.

Stossel, T. P., Hartwig, J. H. and Yin, H. L.

In: Saunders, Daniels, Serrou, Rosenfeld, and Denny (eds,): *Fundamental Mechanisms in Human Cancer Immunology*, New York: Elsevier North Holland, Inc., 1981, pp. 259-273.

Other support: U.S. Public Health Service, the Edwin S. Webster Foundation, Edwin W. Hiam, and C. L. and J. D. Kaufman.

From the Hematology-Oncology Unit, Massachusetts General Hospital, and the Department of Medicine, Harvard Medical School, Boston.

DETERMINANTS OF FORCED EXPIRATORY FLOWS IN NEWBORN INFANTS

This study was undertaken to delineate the mechanical properties of the lung in early life by examining the determinants of forced expiratory flows and the response of flows to HeO_2 in newborn boys and girls. Specifically, maximal flows at functional residual capacity ($Vmax_{FRC}$) from partial expiratory flow-volume (PEFV) curves (achieved with rapid compression of the chest) were obtained on 11 healthy newborn babies. Mean$Vmax_{FRC}$, size corrected by dividing absolute values by measured thoracic gas volume (TGV), was 1.90 TGVs/s. Specific upstream conductances were high, and the cross-sectional area of the flow-limiting segment was estimated to be approximately 0.30 cm^2 in the three infants on whom recoil pressures at FRC were also measured. The cross-sectional area of the major bronchi in the neonate is approximately 0.26-0.30 cm^2. PEFV curves were convex to the volume axis. Many of the neonates increased their flows while breathing a helium-oxygen gas mixture. These results suggest (1) size-corrected flows are higher in the neonate than in older children or adults; (2) the site of the flow-limiting segment at FRC during maximal expiratory maneuvers is in large proximal airways, similar to the adult; and (3) the relationship of airway size to parenchymal size may be similar in neonates and adults or, in fact, airways may be larger, relative to parenchyma, in neonates. Therefore, these physiological data do not support the hypothesis, based on pathological studies, that peripheral airways are disproportionately smaller (when compared with central airways) in infants than in adults.

Taussig, L. M. et al.

Journal of Applied Physiology: Respirat. Environ. Exercise Physiol. 53(5):1220-1227, 1982.

Other support: National Institutes of Health.

From the Department of Pediatrics, Hadassah University Hospital, Mt. Scopus, Jerusalem, Israel.

CAPTOPRIL: ASSOCIATION WITH FETAL DEATH AND PULMONARY VASCULAR CHANGES IN THE RABBIT (41446)

Captopril (D-3-mercapto-2-methylpropanoyl-L-proline) is a rather new, orally effective inhibitor of angiotensin converting enzyme, which has recently been approved for the treatment of refractory systemic hypertension. Administration of this agent has been shown experimentally to decrease circulating levels of angiotensin II and to increase levels of bradykinin and prostaglandins. For the study presented here, the effect of oral captopril on fetal survival was assessed in pregnant adult New Zealand White rabbits (20 treated with captopril and 12 controls). Results showed that fetal death in the treated rabbits was 86%, in contrast to 1% in control rabbits. Some of the rabbits were made hypoxic in a hypobaric chamber (522 mm Hg pressure) during the period of captopril administration. Under these conditions, captopril administered to the maternal rabbits had a demonstrable cardiopulmonary effect in the neonates, as shown by a significant reduction in pulmonary arteriolar medial thickness and both left and right ventricular weights compared to the hypoxic untreated controls. In view of these observations, it would be prudent to avoid using captopril for the treatment of

hypertension during pregnancy, until the mechanism of fetal death and the reasons for species variation are known.

Keith, I.M., *Will, J. A.* and Weir, E. K.

Proceedings of the Society for Experimental Biology and Medicine 170(3):378-383, 1982.

National Institutes of Health.

Other support: From the Departments of Veterinary Science, College of Agricultural and Life Sciences and Anesthesiology, Medical School, University of Wisconsin, Madison, and the Veterans Administration Medical Center, University of Minnesota, Minneapolis.

STANDARDIZATION OF FORMALDEHYDE-INDUCED FLUORESCENCE AND ITS MEASUREMENT TO QUANTIFY SEROTONIN EMISSION IN PULMONARY NEUROENDOCRINE CELLS

The formaldehyde-induced fluorescence (FIF) method is widely used for visualization and quantitative analysis of biogenic amines in tissues. In the paper presented here, a modified and standardized quantitative FIF procedure for producing fluorophores and measuring emission intensity of serotonin-containing neuroepithelial bodies (NEBs) in the rabbit lung is described. This technique, using epifluorescence, was reproduced without significant differences between control groups. Important considerations for reproducibility were: using the same humidity (80% RH) and reaction time (2 hrs.) during the vapor treatment, sectioning at constant relative humidity, avoiding unnecessary heating (sections should not be stretched over a hot plate) and avoiding exposure of sections to light. Optimal emission readings were obtained with sectioning and mounting at 40-50% RH. Readings were reduced by 25% when the mercury light source was switched from 200 W to 100 W. It was also important to let the instruments warm up long enough to avoid drift during quantitation. Each NEB should be subjected to the same duration of light exposure for alignment (30 s) before measuring fluorescence to avoid differences from photodecomposition.

Keith, I. M., Wiley, L. A. and *Will, J. A.*

Histochemistry 75:253-258, 1982.

Other support: College of Agriculture and Life Sciences, University of Wisconsin, Madison.

From the Department of Veterinary Science, University of Wisconsin, Madison.

PLASMA PANCREATIC TRYPSINOGENS IN CHRONIC RENAL FAILURE AND AFTER NEPHRECTOMY

The study presented here had three major objectives: (a) to investigate the relation between plasma pancreatic anionic and cationic trypsinogen in patients with chronic renal failure or nephrectomy, (b) to determine whether hemodialysis alters the plasma levels of these zymogens in patients with chronic renal failure (CRF) or nephrectomy, and (c) to study the effects of nephrectomy on the plasma trypsinogen response to hormonal stimulation of pancreatic zymogen secretion in an established animal model,

the dog. For this study, plasma concentrations of anionic and cationic trypsinogen were measured in CRF and anephric patients. Results showed that the plasma concentrations were significantly elevated in both groups of patients. Hemodialysis did not change their plasma levels. The plasma levels of anionic and cationic trypsinogens were highly correlated in patients and normal subjects; however, the relative concentrations of anionic trypsinogen were significantly higher in renal failure patients. This suggests that in patients with renal failure the secondary clearance mechanisms for these plasma proteins clear cationic molecules more efficiently than they clear anionic molecules. In a related experiment using normal dogs, intravenous infusion of synthetic octapeptide of cholecystokinin (CCK-8) resulted in small transitory increases in plasma trypsinogen levels. After nephrectomy, basal levels of anionic and cationin trypsinogen were elevated, and intravenous infusion of CCK-8 resulted in prolonged, high levels of plasma trypsinogens.

Geokas, M. C. et al.

American Journal of Physiology 242 (Gastrointest. Liver Physiol. 5):G177-G182, 1982.

Other support: Veterans Administration and the National Institutes of Health.

From Martinez Veterans Administration Medical Center, Martinez, CA; Departments of Internal Medicine and Biological Chemistry, University of California Davis School of Medicine, Davis; and the Veterans Administration Wadsworth Medical Center, and Department of Surgery, UCLA School of Medicine, Los Angeles.

III. *Heart and Circulation*

SEROTONIN RECEPTOR-MEDIATED STIMULATION OF BOVINE SMOOTH MUSCLE CELL PROSTACYCLIN SYNTHESIS AND ITS MODULATION BY PLATELET-DERIVED GROWTH FACTOR

This paper reports that serotonin, an indoleamine released from platelet dense bodies, stimulates prostacyclin (PGI_2) production by vascular smooth muscle cells in culture through a specific serotonin-receptor-mediated mechanism. It further reports that serotonin and platelet-derived growth factor (PDGF) act synergistically to elicit dramatic increases in PGI_2 synthesis by vascular smooth muscle cells. In the basic work that led to these conclusions, serotonin (5-hydroxytryptamine: 0.5 μM and above) stimulated the synthesis of PGI_2 (as measured by radioimmunoassay of 6-ketoprostaglandin $F_1\alpha$) by bovine aortic smooth muscle cells in culture. This effect was structurally specific; a similar response was not elicited by the other indoles or by the amines phenylephrine, isoproterenol, dopamine, or histamine. The response was reversible

and was saturable at serotonin concentrations of 10 μM or higher. An increase in PGI_2 synthesis was elicited by the addition of a serotonin agonist, quipazine, and antagonized by the serotonin receptor blockers cyproheptadine, methysergide, or methiothepin. The addition of PDGF to cultures of smooth muscle cells dramatically enhanced PGI_2 synthesis in response to the coadministration of serotonin. PDGF greatly increased the maximum response to serotonin without altering the half-maximal effective concentration for serotonin. Also, this synergistic interaction was blocked by the addition of a serotonin-receptor blocking agent.

Coughlin, S. R., Moskowitz, M. A., *Antoniades, H. N.* and Levine, L.

Proceedings of the National Academy of Sciences of the United States of America 78(11):7134-7138, 1981.

Other support: National Institutes of Health.

From the Departments of Neurosurgery and Neurology, Massachusetts General Hospital, Harvard Medical School, Boston; Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge; Center for Blood Research, Department of Nutrition, Harvard School of Public Health, Boston, and Department of Biochemistry, Brandeis University, Waltham, MA.

HUMAN PLATELET-DERIVED GROWTH FACTOR STIMULATES AMINO ACID TRANSPORT AND PROTEIN SYNTHESIS BY HUMAN DIPLOID FIBROBLASTS IN PLASMA-FREE MEDIA

There are two major points about the action of platelet-derived growth factor (PDGF) on human fibroblasts that emerge from this paper. First, stimulation of amino acid transport and [^{3}H]leucine incorporation are early effects of PDGF action on quiescent human fibroblasts. Second, the actions of PDGF on amino acid uptake and [^{3}H]leucine incorporation are brought about by PDGF alone and do not require simultaneous or subsequent presence of plasma components or other hormones. Specifically, results of this study show that purified human PDGF induces an increase in amino acid uptake via system A in quiescent human diploid fibroblasts. Cells must be exposed to PDGF for 45 min to obtain maximum transport stimulation. Transport stimulation requires protein synthesis; transient exposure to PDGF, alone, in the absence of plasma components can stimulate transport. Acid-insoluble [^{3}H]leucine incorporation is also stimulated by PDGF treatment, and this event also does not require the presence of plasma components. Finally, antiserum to PDGF that blocks PDGF-stimulated DNA synthesis in these cells also blocks PDGF-stimulated amino acid uptake and protein synthesis. Increased amino acid uptake and protein synthesis that occur soon after addition of fresh serum to quiescent cells can be attributed to the action of PDGF acting alone and should be useful as markers for the investigation of early cellular events caused by PDGF.

Owen, A. J. III, Geyer, R. P. and *Antoniades, H. N.*

Proceedings of the National Academy of Sciences of the United States of America 79(10):3203-3207, 1982.

Other support: U. S. Public Health Service and the National Cancer Institute.

From the Department of Nutrition, Harvard School of Public Health, Boston.

A NEW HORMONAL POLYPEPTIDE THAT STIMULATES THE GROWTH OF CELLS IN CULTURE: ISOLATION AND CHARACTERIZATION FROM HUMAN SERUM AND HUMAN PLATELETS

This presentation affords a wide view of the body of Antoniades and his associates. It describes briefly (1) their early studies which led them to the discovery and isolation of a new hormonal polypeptide growth factor from human serum, which appears to be indispensable for the growth of normal cells in culture; (2) the subsequent isolation and purification of this polypeptide from clinically outdated human platelets; (3) the growth effects, and diverse metabolic activities of this purified polypeptide in cells in culture, and (4) studies in progress aimed at the elucidation of its mode of action in the growth of normal cells in culture. In the work described, studies with cell culture and a specific radioimmunoassay demonstrated that the serum polypeptide growth factor derives from platelets and is released into serum during blood clotting; it is not present in platelet-poor plasma. This human platelet-derived polypeptide growth factor (PDGF) has been purified to homogeneity from clinically outdated human platelets. The specific activity of the purified PDGF is 20 million times greater than that found in unfractionated human serum. It stimulates DNA replication and cell proliferation at a concentration of 1 ng/ml. Characterization studies have shown that PDGF is a cationic, heat-stable (100°C) polypeptide, with a pI of about 9.8. The unreduced molecule has an apparent molecular weight of about 35,000 daltons as judged by $NaDodSO_4$/polyacrylamide gel electrophoresis, and reduction results in the production of two inactive polypeptide chains of about 14,000 and 17,000 daltons respectively.

Antoniades, H. N.

In: Bing, D. H. and Rosenbaum, R. A. (eds.): *Plasma and Cellular Modulatory Proteins*, Boston: Center for Blood Research, 1980, pp. 1-14.

Other support: National Cancer Institute.

From the Center for Blood Research and Harvard University School of Public Health, Boston.

HUMAN PLATELET-DERIVED GROWTH FACTOR (PDGF): PURIFICATION OF PDGF-I and PDGF-II AND SEPARATION OF THEIR REDUCED SUBUNITS

In this methodological paper, a procedure that allows the direct recovery of biologically active human platelet-derived growth factor (PDGF) from stained gels after separation on $NaDodSO_4$/polyacrylamide gel electrophoresis is described. This technique enabled the identification and purification to homogeneity of two active PDGF polypeptides, one with a molecular weight of about 35,000 (PDGF-I) and the other with a molecular weight of about 32,000 (PDGF-II). Reduced PDGF-I produced two inactive subunits, with molecular weights of about 15,000 and 18,000. Reduced PDGF-II also produced two inactive subunits, with molecular weights of about 15,000 and 16,000. It is possible that PDGF-II derives from proteolytic cleavage of PDGF-I.

Antoniades, H.N.

Proceedings of the National Academy of Sciences of the United States of America 78(12): 7314-7317, 1981.

Other support: National Institutes of Health.

From the Center for Blood Research and Department of Nutrition, Harvard School of Public Health, Boston.

STIMULATION OF PHOSPHOLIPID AND CHOLESTEROL ESTER SYNTHESIS BY PLATELET-DERIVED GROWTH FACTOR IN NORMAL AND HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA HUMAN SKIN FIBROBLASTS

The aim of this investigation was to study the effect of human platelet-derived growth factor (PDGF) on lipid metabolism, particularly cholesterol ester and phospholipid synthesis in normal and familial hypercholesterolemic-derived human skin fibroblasts. Results of this study show that pure PDGF at nanogram levels stimulates cholesterol ester, phospholipid and DNA synthesis in both normal and familial hypercholesterolemia mutant human skin fibroblasts. While stimulation of DNA synthesis did not begin until 15-24 hrs. after addition of PDGF to quiescent normal and FH mutant fibroblasts, stimulation of [^{3}H]oleic acid incorporation into cholesterol ester and phospholipid was evident 3-6 hrs. after the addition of PDGF. In the normal cells, the rate of cholesterol ester synthesis was maximal at 24 hrs., then rapidly declined. Cholesterol esterification was much lower in the FH cells than in the normal cells. The stimulation of [^{3}H]oleic acid incorporation into cholesterol ester by PDGF was inhibited in both normal and FH mutant skin fibroblasts by progesterone, an inhibitor of acyl-CoA: cholesterol acyltransferase. The rate of cholesterol ester synthesis in the normal cells increased as the concentration of platelet-poor plasma or low density lipoprotein (LDL) was increased, especially in the presence of PDGF. Linearization of the LDL dose-response curve indicated that PDGF increased the rate rather than the affinity of the overall cholesterol esterification system. The rate of cholesterol esterification in the FH mutant cells was highest in the absence of LDL or at low levels of platelet-poor plasma. Consequently, PDGF can stimulate cholesterol ester synthesis by LDL and non-LDL-mediated processes.

Leslie, C. C., *Antoniades, H. N.* and Geyer, R. P.

Biochimica et Biophysica Acta 711:290-304, 1982.

From the Department of Nutrition, Harvard School of Public Health, Boston.

MIGRATION OF CULTURED VASCULAR CELLS IN RESPONSE TO PLASMA AND PLATELET-DERIVED FACTORS

In the study presented here, a quantitative assay for cell migration was used to measure the response of pericytes, smooth muscle cells and endothelial cells from large and small vessels to human serum, plasma and purified platelet-derived factors.

In the first part of this study, phagokinetic migration of cultured vascular cells was tested in response to human platelet-rich serum ('serum') and human platelet-poor plasma serum ('plasma'). The cell types tested included bovine aortic endothelial cells, human umbilical vein endothelial cells, human hemangiomal capillary endothelial cells, bovine adrenal microvascular pericytes, and bovine aortic smooth muscle cells. Human serum stimulated a significant increase in the rate of migration in all five cell types. Human plasma stimulated the endothelial cells to migrate but had no effect on the migration of pericytes or smooth muscle cells. In the second part of this study, highly purified platelet-derived growth factor (PDGF) stimulated dose-dependent migration of smooth muscle cells causing a 50% increase in phagokinetic track area relative to controls. Neither pericyte nor endothelial cell migration was stimulated by PDGF. Rabbit antiserum to human PDGF completely blocked the smooth muscle cell migration induced by either 10% serum of lng/ml pure PDGF. Purified platelet factor IV (PF_4) stimulated migration of pericytes but not of smooth muscle cells or endothelial cells. Sheep antiserum to human PF_4 completely blocked the pericyte migration induced by either 10% serum or 1 ug/ml pure PF_4. These results indicate that PDGF is the primary factor in serum responsible for the migration of cultured aortic smooth muscle cells and that PF_4 is a critical factor required to induce the migration of pericytes. Other factors present in both plasma and serum control the migration of vascular endothelial cells.

Bernstein, L. R., *Antoniades, H.* and Zetter, B. R.

Journal of Cell Science 56:71-82, 1982.

Other support: National Institutes of Health.

From the Departments of Physiology and Surgery, Harvard Medical School, Children's Medical Center, and Harvard School of Public Health, Center for Blood Research, Boston.

PLATELET-DERIVED GROWTH FACTOR BINDS SPECIFICALLY TO RECEPTORS ON VASCULAR SMOOTH MUSCLE CELLS AND THE BINDING BECOMES NONDISSOCIABLE

Platelet-derived growth factor (PDGF) is a potent stimulant of the growth of vascular smooth muscle. In order to understand the action of PDGF, methods had to be developed for identifying PDGF receptor sites. To accomplish this, radioiodinated PDGF (^{125}I-PDGF) was used in studies of PDGF binding sites on vascular smooth muscle cells. There was an excellent correlation between the ability of ^{125}I-PDGF to stimulate cell proliferation and to bind specifically to cultured vascular smooth muscle cells. The half-maximal concentration for both processes was 0.1 nM. There were 50,000 binding sites per cell. Reduced PDGF, prepared by treatment of PDGF with 20 mM dithiothreitol, had neither the ability to bind to smooth muscle cells nor to stimulate cellular proliferation. Epidermal growth factor, nerve growth factor, fibroblast growth factor, and histone B did not compete for the binding sites at a concentration of 10 nM. ^{125}I-PDGF binding was slowly reversible at 4°C and was rapidly and totally

reversible after a 1-min incubation at 37°C. After continued incubation at 37°C, the binding became irreversible. The half-time for formation of the nondissociable state of ^{125}I-PDGF was ≈ 5 min at 37°C. The nondissociable state of binding was not formed at 4°C even after 1 hr of incubation. These data suggest that the labeled sites are the PDGF receptors that mediate PDGF's mitogenic action and that a nondissociable state of PDGF binding is formed at 37°C. It is likely that nondissociable PDGF represents internalized ligand or binding to sites that are converted to a high-affinity state after the ligand binds.

Williams, L. T., Tremble, P. and *Antoniades, H. N.*

Proceedings of the National Academy of Sciences of the United States of America 79:5867-5870, 1982.

Other support: National Institutes of Health and the Milton Fund of Harvard Medical School.

From the Cardiac Unit, Massachusetts General Hospital, Boston, and the Center for Blood Research and Department of Nutrition, Harvard University, School of Public Health, Boston.

CONTRIBUTION OF PLASMA PROTEASE INHIBITORS TO THE INACTIVATION OF KALLIKREIN IN PLASMA

This study was designed to assess the respective contribution of the different plasma protease inhibitors to the inactivation of kallikrein in plasma. The use of a new technique that selectively inactivates α_1M in plasma facilitated the comparison of the kinetics of kallikrein inactivation. Results showed that, under pseudo-first-order conditions, the inactivation rate constant of kallikrein in normal plasma was 0.60 min^{-1}. This rate constant was reduced to 0.35, 0.30, and 0.06 min^{-1}, in plasma deficient respectively in C$\bar{1}$-INH, α_2M, or both inhibitors. Thus C$\bar{1}$-INH (42%) and α_2M (50%) were found to be the major inhibitors of kallikrein in normal plasma. Moreover, all the other protease inhibitors present in normal plasma contributed only for 8% to the inactivation of the enzyme. To confirm these kinetic results, ^{125}I-Kallikrein (M, 85,000) was completely inactivated by various plasma samples, and the resulting mixtures were analyzed by gel filtration on Sepharose 6B CL for the appearance of ^{125}I-kallikrein-inhibitor complexes. Overall, the results presented here indicate that C$\bar{1}$-INH and α_2M are the major inhibitors of kallikrein in normal human plasma. This conclusion is supported by (a) the analysis of the kinetics of kallikrein inactivation in normal and protease inhibitor-deficient plasma, and (b) by the quantitation upon gel filtration of the kallikrein-inhibitor complexes formed in plasma as the result of the inactivation of purified radiolabeled enzyme.

Schapira, M., Scott, C. F. and *Colman, R. W.*

Journal of Clinical Investigation 69:462-468, 1982.

Other support: National Institutes of Health.

From the Thrombosis Research Center, Temple University School of Medicine, Philadelphia.

HIGH MOLECULAR WEIGHT KININOGEN OR ITS LIGHT CHAIN PROTECTS HUMAN PLASMA KALLIKREIN FROM INACTIVATION BY PLASMA PROTEASE INHIBITORS

Five plasma protease inhibitors are known to inactivate kallikrein, but now it has been shown also that high molecular weight kininogen (HMWK) or its light chain can act to protect kallikrein from inactivation by these inhibitors. For this biochemical study, the kinetics of the inactivation of kallikrein by α_2-macroglobulin, antithrombin III, and α_1-antitrypsin were noted in the presence and absence of HMWK. In the absence of HMWK, the second-order rate constants k_{+2}/K_i for the inactivation were respectively 6.9×10^3, 1.8×10^4, and $2.5 \times 10^2 M^{-1}min^{-1}$. When HMWK was present during the reaction, the inactivation rates by these plasma protease inhibitors were reduced as a result of the formation of kallikrein-high molecular weight kininogen complex, $K_d = 0.75$ μM. When the light chain derived from reduction of kinin-free HMWK was substituted for the parent molecule, a more pronounced reduction in inactivation rates was observed due to the formation of a kallikrein-light chain complex ($K_d = 0.14$ μM). These results demonstrate that the combining site for kallikrein on HMWK, responsible for complex formation and protection against inhibitors, resides in the light-chain region of the molecule. Moreover, kallikrein appears to bind more tightly to the light chain of HMWK than to the parent molecule.

Schapira, M., Scott, C. F., James, A., Silver, L. D., Kueppers, F., James, H. L., and *Colman, R. W.*

Biochemistry 21(3):567-572, 1982.

Other support: National Institutes of Health.

From the Thrombosis Research Center and the Department of Medicine, Temple University Health Sciences Center, Philadelphia.

REGIONAL RENAL AND SPLANCHNIC BLOOD FLOWS DURING NICOTINE INFUSION: EFFECTS OF *ALPHA* AND OF COMBINED *ALPHA* AND *BETA* ADRENERGIC BLOCKADE

This study was instituted for the purpose of defining directly the roles of *alpha* adrenergic and possible nonadrenergic mechanisms in vasoconstrictor responses to nicotine. To do this, nicotine-induced changes in blood flow and vascular conductance in regional renal and splanchnic beds were evaluated before and after (1) selective blockade of *alpha* adrenergic receptors and (2) combined blockade of *alpha* and *beta* adrenergic receptors. Before adrenergic blockade, nicotine increased arterial pressure (+82%) but had heterogeneous directional effects on regional blood flows: pancreas (−64%), duodenum (−33%), kidney cortex (−31%), kidney medulla (−17%), liver (+5%) and spleen (+71%). Vascular conductance was reduced in kidney cortex, kidney medulla, duodenum, liver and pancreas, and was not altered in spleen. Selective *alpha* adrenergic blockade prevented the hypertensive response to nicotine, but heterogeneous changes in regional flows persisted. After combined *alpha* and *beta* adrenergic blockade, nicotine increased systemic arterial pressure and decreased

vascular conductance in all tissues. Results indicate that there are (1) a heterogeneous influence of nicotine in renal and splanchnic circulations associated with regional differences in activities of *alpha* and *beta* adrenergic receptors and (2) a potent non-adrenergic vasoconstrictor response in these circulations to nicotine after blockade of *alpha* and *beta* adrenergic receptors.

Downey, H. F., Crystal, G. J. and Bashour, F. A.

The Journal of Pharmacology and Experimental Therapeutics 220(2):375-381, 1982.

Other support: Cardiology Fund.

From the Departments of Physiology and Internal Medicine, University of Texas Health Science Center and Cardiovascular Research Laboratory at Methodist Hospital, Dallas.

EFFECT OF CIGARETTE SMOKING ON HIGH DENSITY LIPOPROTEIN PHOSPHOLIPIDS

This study was designed to assess the effect of acute inhalation of cigarette smoke on high density lipoprotein (HDL) phospholipid composition. In running the tests on White Carneau pigeons, the following four treatment regimens were instituted: (1) shelf control birds fed a chow diet and retained in their cages; (2) sham pigeons fed a cholesterol-saturated fat diet and exposed to fresh air by a smoking machine; (3) low nicotine-low carbon monoxide (LoLo) animals also fed the cholesterol-fat diet and exposed to low concentrations of these cigarette smoke products; and (4) high nicotine-high carbon monoxide (HiHi) birds fed the cholesterol-fat diet and subjected to high concentrations of these inhalants. Results of these studies showed that the cholesterol diet caused an increase in the concentration of most HDL phospholipid classes. Exposure to the HiHi regimen resulted in an increase in the HDL cholesterol/phospholipid ratio and a reduction in the concentration of HDL phosphatidyl ethanolamine, phosphatidyl serine/inositol, sphingomyelin and lysophosphatidyl choline. It appears, therefore, that cigarette smoking may attenuate HDL's anti-atherogenic properties by altering surface phospholipid components.

Hegarty, K. M., Turgiss, L. E., Mulligan, J. J., Cluette, J. E., Kew, R. R., Stack, D. J., and *Hojnacki, J. L.*

Biochemical and Biophysical Research Communications 104(1):212-219, 1982.

Other support: American Heart Association, Greater Boston Division.

From the Department of Biological Sciences, University of Lowell, Lowell, MA.

ALTERNATIVE COMPLEMENT PATHWAY-DEPENDENT INGESTION OF FLUOLITE PARTICLES BY HUMAN GRANULOCYTES

Particles of a fluorescent cyclic hydrocarbon (Fluolite) have been reported before to be ingested by mononuclear phagocytes of human blood. This paper describes

quantitative studies of the ingestion of these same particles by human granulocytes. In the studies presented here, Fluolite particles with an average size of 0.1μM were ingested by human granulocytes after incubation in fresh normal human serum. Ingestion, which was assessed by visual counting in a fluorescent microscope of cells containing particles, required fresh normal serum and did not occur when serum was heated for 30 minutes at 50°C or when ethylenediaminetetraacetic acid (EDTA) was present. Particularly, it did not occur in serum genetically deficient in C3b inactivator or in C3. However, phagocytic activity was restored to C3-deficient serum by purified human C3 and to heat inactivated serum by purified factor B. Opsonization of particles under appropriate conditions is insensitive to the absence of human C2 or C5, but is dependent upon C3 and an intact alternative C pathway. Measurement of the opsonization of these particles thus constitutes a simple assay of the functional integrity of this pathway. Although the mechanism involved here is still unclear, this simple and readily available assay can be used clinically as a screen for the functional opsonic activity of the alternative C pathway, as well as for the capacity of human granulocytes to recognize and ingest C3-coated particles.

Arnaout, M. A., Luscinskas, F. W., *Lionetti, F. J.*, Alper, C. A., and Valeri, C. R.

The Journal of Immunology 127(1):278-281, 1981.

Other support: Office of Naval Research.

From the Division of Cell Biology and Nephrology, Children's Hospital Medical Center, Boston; Center for Blood Research, Boston; and Naval Blood Research Laboratory, Boston.

ENDOTHELIAL METABOLISM

There are three important functions of endothelium—(1) separation of blood and lymph from the extravascular space, (2) control of the influx and efflux of specific blood solutes and colloids, and (3) provision of a smooth gliding surface for passing blood—that have been well recognized for years. The book chapter presented here, though, deals not with these three functions but with the metabolic activities of the endothelium. While endothelial cells are well-equipped for glycolysis, oxidative phosphorylation and the complex series of reactions required for cell division, it is the number of far-reaching metabolic activities, most discovered since 1968, that are considered here. To wit, endothelial cells possess a highly complex array of intracellular machinery and can, in fact, elaborate molecules and macromolecules important not only to cell integrity and cell division but also important in terms of processing vasoactive and hemostatic substances. Many of the recently recognized metabolic activities occur at or near the cell surface. For example, angiotensin-converting enzyme appears to be disposed so that the enzyme itself is embedded in the plasma membrane, yet its catalytic site is situated to have access to angiotensin I and bradykinin as they pass in circulating blood. One section of this paper deals with anatomical considerations, and another with surveying the endothelium's known metabolic activities. While much of the work on endothelial metabolism performed so far has been accomplished through the disciplines of biochemistry, morphology, and cell biology,

other studies, physiologic, pharmacologic, and pathologic, are feasible, and there is every reason to believe that clinically relevant data will be forthcoming.

Ryan, J. W. and *Ryan, U. S.*

In: Wiedman, M. P. (ed.): *An Introduction of Microcirculation*, New York: Academic Press, Inc. 1981, pp. 147-169.

Other support: U.S. Public Health Service and the John A. Hartford Foundation, Inc.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL.

STRUCTURAL BASES FOR METABOLIC ACTIVITY

The pulmonary endothelium, which is the first line of contact between blood-borne substrates and the cellular machinery of the lungs, is reviewed here along with those structural specializations that are suited for their role in the metabolic functions of the lungs. The underlying structural bases for metabolic activity are considered here under the three headings: anatomical, cellular, and subcellular. Particular emphasis is placed upon the enzymes, inhibitors, receptors, and transport mechanisms that are important in the regulation of the hormonal composition and fluidity of blood. Following anatomical study, a picture emerges of a variety of enzymes that are situated on the surface of pulmonary endothelial cells and are strategically poised for interaction with the appropriate substrates delivered by the blood and are equally strategically placed to determine the quantities of active substances allowed to pass downstream or into the extravascular space. Cellular studies show that the caveolae-plasma membrane fraction of lung homogenate converts angiotensin I to angiotensin II and degrades bradykinin to yield the characteristic products formed by intact lungs. Furthermore, the preparation can degrade ATP and 5′-AMP to adenosine and free phosphate. By use of a sensitive radioassay, it was also shown that pulmonary endothelial cells contain abundant angiotensin converting enzyme (ACE). In summary, it appears that the unique features of pulmonary enzymes may relate to the structure of the lungs and to the position of the lung in the circulatory system. Thus, the total amount of ACE within the lungs may greatly exceed the amount needed to process the concentration of angiotensin I and bradykinin usually found in pulmonary artery blood. While it is known that ACE occurs on the luminal surface of these cells, it is becoming evident at a subcellular level that the membrane bound enzyme is sensitive to factors such as oxygen concentration. However, the effects of components of the plasma membrane on the molecular configuration and hence on the activity of the enzyme are not known as yet.

Ryan, U. S.

Annual Review of Physiology 44:223-239, 1982.

Other support: National Institutes of Health.

From the Department of Medicine, University of Miami School of Medicine, Miami, FL.

NEUTROPHILS ARE REQUIRED FOR THE DNA SYNTHETIC RESPONSE OF HUMAN LYMPHOCYTES TO MEVALONIC ACID: EVIDENCE SUGGESTING THAT A NONSTEROL PRODUCT OF MEVALONATE IS INVOLVED

The ability of various enantiomeric forms of mevalonic acid to initiate lymphocyte DNA synthesis is examined in this paper, and the role of the neutrophilic polymorphonuclear leukocyte as the helper cell in evoking mevalonate-induced lymphocyte proliferation is defined with greater precision than has been accomplished before. The ability of lymphocytes to initiate DNA synthesis and cell cycling is a radiosensitive property of the cells, whereas the help provided by neutrophils is maintained despite their prior exposure to x-irradiation. Other organic acid anions, including precursors of mevalonic acid biosynthesis and a variety of products of mevalonate metabolism, fail to initiate DNA synthesis when added to human lymphocytes. Because only the metabolically active R(−) enantiomer of mevalonic acid initiates lymphocyte DNA synthesis, it seems probable that physiological pathways of mevalonate metabolism are involved. The response to mevalonic acid of ML-236B (compactin)-inhibited lymphocytes is increased, and the threshold concentration of mevalonate at which lymphocyte DNA synthesis first appears is decreased, when the cells are cultured in lipoprotein-containing medium. The response to mevalonic acid of lymphocytes cultured in lipoprotein-depleted medium can be enhanced by addition to the cultures of low density lipoprotein but not by addition of high density lipoprotein. Based upon the flux diversion hypothesis of mevalonate metabolism, these observations suggest that a nonsterol product of mevalonate metabolism may be responsible for the initiation of lymphocyte DNA synthesis by mevalonic acid.

Larson, R. A., Chung, J., Scanu, A. M., and *Yachnin, S.*

Proceedings of the National Academy of Sciences of the United States of America 79:3028-3032, 1982.

Other support: U.S. Public Health Service and the Nalco Center Research Fund.

From the Departments of Medicine and Biochemistry, the Franklin McLean Memorial Research Institute, and the Committee on Immunology, The University of Chicago School of Medicine, Chicago.

MEVALONIC ACID IN CONJUNCTION WITH HELP FROM NEUTROPHILS INDUCES DNA SYNTHESIS AND CELL CYCLING IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES

Mevalonic acid plays an important role in the regulation of mammalian cell growth and division. Results from earlier studies have even suggested a critical role for mevalonic acid, independent of its conversion to cholesterol, in the regulation of DNA synthesis and cell replication. Evidence presented in this paper shows that mevalonic acid does stimulate DNA synthesis in human peripheral blood lymphocytes which have been isolated by gravity sedimentation of blood and freed of adherent cells by nylon column passage. Human peripheral blood mononuclear cells isolated by the Ficoll-Hypaque technique respond less well, but their response to mevalonic acid can be enhanced by the neutrophil-rich Ficoll-Hypaque-isolated "bottom" cell fraction. The kinetics of mevalonic acid-induced lymphocyte transformation are similar to those of more classic lymphocyte mitogens. In addition to stimulating lymphocyte DNA synthesis, mevalonic acid produces a population of cells representing all phases of the cell

cycle whose morphological characteristics are typical of those seen with more conventional mitogens. The DNA synthetic response of lymphocytes to mevalonic acid can be abolished by prior exposure of the lymphocytes to x-irradiation or mitomycin C, while the helper effect of granulocytes is unaffected by either treatment. These observations suggest that mevalonic acid not only may play a role as a critical substance which supports the propogation of cells programmed to divide, or stimulated to divide by various initiators of cell growth, but also that, in susceptible cell populations, mevalonic acid may act as an inducer of the entire program of the cell cycle.

Yachnin, S. and Richman, D. P.

Cellular Immunology 72:248-262, 1982.

Other support: U.S. Public Health Service, Muscular Dystrophy Association, and the Nalco Research Foundation.

From the Department of Medicine and Neurology, the Franklin McLean Memorial Research Institutes, and the Committee on Immunology, The University of Chicago School of Medicine, Chicago.

IV. *Neuropharmocology and Physiology*

NICOTINE BINDING SITES AND THEIR LOCALIZATION IN THE CENTRAL NERVOUS SYSTEM

The study of nicotine binding in the brain, which was plagued with problems during earlier investigations, has been helped greatly by the development of a method of obtaining the unnatural (+)-isomer optically pure and by the refined synthesis of radiolabeled nicotine with high specific activity. The actions of the stereoisomers of nicotine on the central nervous system are qualitatively similar in most tests but (−)-nicotine is more potent than the unnatural (+)-isomer by at least 10-fold. Binding of radiolabeled nicotine to brain has both saturable and nonsaturable components. Only saturable binding is affected by incubation conditions such as time, temperature, pH and ion concentration. Excess concentrations of the stereoisomers are equally effective in displacing (−)-[^{3}H]-nicotine from brain homogenates. Nevertheless, a direct comparison of (+)-[^{3}H]-nicotine and (−)-[^{3}H]-nicotine binding shows that the latter has a K_D three times lower than the former. (−)-[^{3}H]-nicotine is bound to the greatest degree in hypothalamus and hippocampus, areas that also exhibit the most stereoselectivity for nicotine. However, differences in the binding affinities of the two isomers are far less than the pharmacological stereospecificity observed.

Martin, B. R. and *Aceto, M. D.*

Neuroscience & Behavioral Reviews 3(4):473-478, 1981.

Other support: National Institutes of Health.

From the Department of Pharmacology, Medical College of Virginia, Richmond.

CHARACTERIZATION OF NICOTINE BINDING IN MOUSE BRAIN AND COMPARISON WITH THE BINDING OF α-BUNGAROTOXIN AND QUINUCLIDINYL BENZILATE

The literature suggests that nicotine binds to neuronal tissue, but the nature and significance of this binding are in question. In this study of nicotine binding, the binding of [^{3}H]nicotine to mouse brain was measured and subsequently compared with the binding of [^{125}I]α-bungarotoxin (α-BTX) and L-[^{3}H] quinuclidinyl benzilate (QNB). The binding of nicotine was saturable, reversible and stereospecific. The average K_D and B_{max} were 59 nM and 88 fmoles/mg of protein, respectively. Although the rates of association and dissociation of nicotine were temperature-dependent, the incubation temperature had no effect on either K_D or B_{max}. When measured at 20° or 37°, nicotine appeared to bind to a single class of binding sites, but a second, very low-affinity, binding site was observed at 4°. Nicotine binding was unaffected by the addition of NaCl, KCl, $CaCl_2$, or $MgSO_4$ to the incubation medium. Nicotine cholinergic agonists were potent inhibitors of nicotine binding; however, nicotine antagonists were poor inhibitors. The regional distribution of binding was not uniform: midbrain and striatum contained the highest number of receptors, whereas cerebellum had the fewest. When nicotine, α-BTX, and QNB binding were compared in several ways, results indicated that all three ligands label cholinergic sites, but these sites differ from one another. Differences in site densities, regional distribution, inhibitor potencies, and thermal denaturation indicated that nicotine binding was not the same as either QNB or α-BTX binding, and therefore that receptors for nicotine may represent a unique population of cholinergic receptors.

Marks, M. J. and *Collins, A. C.*

Molecular Pharmacology 22:554-564, 1982.

Other support: National Institutes of Health.

From the Institute for Behavioral Genetics and School of Pharmacy, University of Colorado, Boulder.

EFFECTS OF ACUTE CENTRAL AND PERIPHERAL ADMINISTRATION OF NICOTINE ON HYPOTHALAMIC CATECHOLAMINE NERVE TERMINAL SYSTEMS AND ON THE SECRETION OF ADENOHYPOPHYSEAL HORMONES IN THE MALE RAT

The actions of intraventricular injections and intravenous infusions of nicotine were evaluated on dopamine and noradrenaline stores and turnover in discrete hypothalamic dopamine and noradrenaline nerve terminal systems in male Sprague-Dawley rats. Anterior pituitary hormone secretion was also studied in the same group of animals. Specifically, measurements were made of LH, FSH, GH, TSH, prolactin and corticosterone serum levels using radioimmunological procedures. Intraindividual correlations between regional catecholamine levels and turnover and hormone secretion were performed. Results showed that intraventricular injections of nicotine produced dose-dependent reductions of dopamine and noradrenaline levels and increases of dopamine and noradrenaline turnover in discrete hypothalamic areas. The turnover

increases were associated with reductions of serum levels of TSH, prolactin and LH and an increase of serum FSH levels. Correlation analysis in the intraventricular experiments was performed. Also, intravenous infusions of nicotine over one hour produced dose-dependent reduction of dopamine and noradrenaline concentrations and increased the turnover of these monoamines in the various hypothalamic catecholamine nerve terminal systems analyzed, with the median eminence, dopamine and noradrenaline nerve terminal systems showing the highest sensitivity to nicotine. Overall, the total of these and other experimental findings reinforces the view that nicotine may act directly on the brain to activate dopamine and noradrenaline nerve terminal systems, probably via activation of nicotine-like cholinergic receptors. Correlation analysis shows that other neurotransmitter mechanisms must also be involved in producing the nicotine-induced changes in the secretion of anterior pituitary hormones.

Andersson, K., *Fuxe, K.*, Eneroth, P., and Agnati, L. F.

Medical Biology 60:98-111, 1982.

Other support: Svenska Tobaks AB, Stockholm.

From the Department of Histology, Karolinska Institutet, and the Hormone Laboratory, Karolinska Hospital, Stockholm: and the Department of Human Physiology, University of Modena, Modena, Italy.

ON THE INTERACTION BETWEEN NICOTINE AND CYCLOHEXIMIDE

Inhibitors of protein synthesis, such as cycloheximide, have been shown to affect memory retention and nicotine has been shown to reverse the amnestic properties of cycloheximide. In the study presented here, the interactions of nicotine and cycloheximide on brain protein synthesis were examined in an attempt to determine whether the nicotine-induced reversal of the amnestic effect of cycloheximide is due to an interaction between nicotine and cycloheximide on brain protein synthesis, and whether it involves the sites in brain that saturably bind [^{3}H]nicotine. Results showed that nicotine did not reverse the cycloheximide-induced inhibition of protein synthesis, both *in vivo* (intact animal) and *in vitro* (brain slices), suggesting that on-going protein synthesis is not necessarily involved in memory consolidation. Also, the nicotine binding sites were not affected by *in vivo* or *in vitro* treatment with cycloheximide either in the presence or absence of nicotine.

Sershen, H., Reith, M. E. A. and *Lajtha, A.*

Brain Research 251:183-185, 1982.

From the Center for Neurochemistry, Rockland Research Institute, Ward's Island, NY.

EVIDENCE FOR A NONCHOLINERGIC NICOTINE RECEPTOR ON HUMAN PHAGOCYTIC LEUKOCYTES

Radioligand binding studies show that leukocytes contain a number of peptide and hormone receptors, such as those for chemotactic peptides and acetylcholine. In this

attempt to test the hypothesis that nicotine acts as a receptor (known or unknown), the specific binding of ^{3}H-(d,l) nicotine to leukocytes was measured. Results of this study demonstrate the presence of a noncholinergic nicotine receptor on human phagocytic leukocytes. The average affinity +/− standard deviation of (d,l)-nicotine for the receptor on neutrophils is 36 +/− 18 nM (n=6). The binding is saturable with an average of 8.7×10^4 sites per neutrophil. Monocytes and, to a lesser extent, lymphocytes but not erythrocytes also display specific binding. Bound nicotine is dissociable from the receptor and is not metabolized. Only close structural analogs of nicotine bind to the receptor, which is stereoselective for the (d)-isomer. The receptor can be occupied by (1)-nicotine at concentrations present in the blood of smokers. It is suggested that some of the adverse effects of smoking on leukocyte functions may be mediated by a specific nicotine receptor.

Davies, B. D., *Hoss, W.*, Lin, J-P., and Lionetti, F.

Molecular and Cellular Biochemistry 44:23-31, 1982.

Other support: U. S. Public Health Service.

From the Center for Brain Research, University of Rochester School of Medicine and Dentistry, Rochester, NY, and Center for Blood Research, Boston.

CHARACTERIZATION OF THE ISOLATED PERFUSED MOUSE BRAIN AS A SYSTEM FOR NEUROCHEMICAL STUDIES

The criteria for characterization of isolated perfused brain preparations include the demonstration of electrical, structural and metabolic viability. The purpose of this study was to meet these criteria and to establish the isolated perfused mouse brain (IPMB) as a viable model for neurochemical and neuropharmacological studies. In the paper presented here, the preparation of the IPMB is described along with its electrophysiological, morphological, biochemical and pharmacological properties. Using high performance liquid chromatography with electrochemical detection, the primary metabolite of mammalian central nervous system norepinephrine, 3-methoxy-4-hydroxyphenethyleneglycol (MHPG), was measured in the perfusate at 15-min. intervals. The rate of MHPG production was similar to literature values of the rate of norepinephrine turnover in mouse brain. MHPG production rate in the IPMB was blocked by pretreatment with 6-hydroxydopamine and was increased by pretreatment with reserpine.

Towell, J. F. and *Erwin, V. G.*

Brain Research 209:476-481, 1981.

Other support: U. S. Public Health Service and the University of Colorado National Center for Alcohol Research.

From the School of Pharmacy, University of Colorado, Boulder.

ANALYSIS OF REGIONAL VARIATIONS IN THE AFFINITIES OF MUSCARINIC AGONISTS IN THE RAT BRAIN

The specific binding of radiolabeled antagonists has been used to characterize the muscarinic acetylcholine receptor in brain. For the study reported here, the affinities of

muscarinic agents in brains from male Sprague-Dawley rats were determined by direct and indirect assays of binding to the receptor. Results showed that the brain stem of the rat has a higher affinity toward muscarinic agonists than does the forebrain, Receptor occupancy curves of both regions of the brain deviate from simple mass-action binding. The characteristics of the binding in each region are compatible with the existence of two non-interacting binding sites, and are not attributable to desensitization or to negatively cooperative binding within a small oligomer; however, the possibility of large oligomers remains to be excluded. The agonist binding data were analyzed by a linear transformation of Scatchard-like inhibition curves of the binding of the antagonist [^{3}H]quinuclidinyl benzilate. Such analysis, based on model of two subpopulations of receptors in each area, shows the subpopulations of the brain stem and the forebrain to be distinct. Brain stem: 44% of receptors possess high affinity with dissociation constant for carbachol, $K_H = 2.8 \times 10^{-6}$ M, dissociation constant of low-affinity receptor, $K_L = 2.3 \times 10^{-6}$ M; forebrain: 41% high affinity, $K_H = 2.1 \times 10^{-7}$ M, $K_H = 1.7 \times 10^{-5}$ M. The data suggest that whole brain contains at least three major muscarinic receptors which can be distinguished on the basis of their affinities for agonists.

Ellis. J. and *Hoss, W.*

Brain Research 193:189-198, 1980.

Other support: National Institutes of Health.

From the Center for Brain Research, University of Rochester School of Medicine and Dentistry, Rochester, NY.

COMPETITIVE INTERACTION OF GALLAMINE WITH MULTIPLE MUSCARINIC RECEPTORS

Neural membranes from the brains of male Sprague-Dawley rats were prepared and used for binding studies with gallamine and carbachol. In the paper presented here, it is shown that gallamine, a nicotinic antagonist with antimuscarinic potency in several systems, interacts competitively with the tritiated ligand [^{3}H]quinuclidinyl benzilate (QNB) at the muscarinic receptor. The occupancy curves derived from these studies suggest that gallamine has widely varying affinities for different subpopulations of muscarinic receptors, a finding which sets gallamine apart from classical muscarinic antagonists such as atropine and QNB. The greatest difference in affinities for gallamine occurred in the brain stem, where the data could be satisfactorily fitted to a two-site model, with 77% of the receptors having high affinity ($K_d = 25$ nM) and 23% low affinity (93 μM). Further, these affinities displayed rank order correlating with those of carbachol (an agonist), although gallamine has not, so far, displayed agonist (or partial agonist) activity. The finding that antagonists as well as agonists can display multiple affinities for muscarinic receptors suggests that there are fundamental differences among subpopulations of these receptors.

Ellis, J. and *Hoss, W.*

Biochemical Pharmacology 31(5):873-876, 1982.

Other support: National Institutes of Health.

From the Center for Brain Research, University of Rochester School of Medicine and Dentistry, Rochester, NY.

V. *Pharmacology and Biochemistry*

AN ISOLATED PERFUSED DOG-LUNG PREPARATION FOR THE STUDY OF CYCLIC GMP METABOLISM: EFFECTS OF SODIUM NITROPRUSSIDE AND OXYGEN

In recent years, considerable interest has developed in the role that cyclic GMP may play in pulmonary function, partly because the lung has a high guanylate cyclase activity compared with other tissues and partly because the lung is in direct contact with various environmental pollutants, many of which are known to precipitate the formation of oxygen free radicals and damage the lung. For the study presented here, the intact, isolated perfused dog lung was evaluated as a model for studies directed at defining the role of oxidative modulation of lung cyclic GMP metabolism in pulmonary function. Sodium nitroprusside added to the perfusion blood increased the cyclic GMP content of lung over four-fold in a dose-dependent manner. Although sodium nitroprusside administration caused changes in lung vascular resistance, these occurred independently of the changes in cyclic GMP. Ventilation of lungs with a high oxygen gas mixture containing 95% 0_2, 5% CO_2 acutely increased the cyclic GMP content of lungs after 15 minutes from 1.3 ±0.06 to 3.4 ±0.12 pmol cyclic GMP/mg protein. Cyclic GMP levels returned toward control during continued ventilation with the high oxygen concentration. The oxygen-induced elevation of lung cyclic GMP content was not accompanied by changes in lung vascular resistance. The results indicate that the isolated perfused lung may be useful in studies of cyclic GMP, tissue oxidation and pulmonary function.

Braughler, J. M. and Maron, M. B.

European Journal of Pharmacology 78:187-193, 1982.

Other support: American Lung Association.

From the Programs in Pharmacology and Physiology, Northeastern Ohio Universities College of Medicine, Rootstown.

INVOLVEMENT OF SULFHYDRYL GROUPS IN THE OXIDATIVE MODULATION OF PARTICULATE LUNG GUANYLATE CYCLASE BY NITRIC OXIDE AND N-METHYL-N-NITRO-NITROSOGUANIDINE

This report describes the activation of particulate rat lung guanylate cyclase by nitric oxide and N-methyl-N-nitro-N-nitrosoguanidine (MNNG) and suggests the involvement of sulfhydryl groups in the activation process. In this study, particulate guanylate cyclase from rat lung was activated by nitric oxide or MNNG in a dose-dependent manner that was affected by dithiothreitol. Although low concentrations of nitric oxide or MNNG produced near maximum activation, excessive amounts decreased the particulate enzyme activity. Nitric oxide-stimulated guanylate cyclase activity decayed during a 60-minute preincubation period at 37°C, but did not decay at 24° or 4°. Dithiothreitol enhanced the decay of nitric oxide-stimulated enzyme at all temperatures by potentiating the reversal of nitric oxide activation. Following the reversal of nitric oxide activation at 24° by dithiothreitol, the particulate enzyme could

be reactivated by a second exposure to nitric oxide. Preincubation of basal particulate guanylate cyclase activity at 37° resulted in the loss of enzyme responsiveness to activation by nitric oxide or MNNG. This loss of responsiveness that was prevented by the thiol antioxidants was potentiated by the thiol oxidants, diamide or oxidized glutathione. Also, the inhibitory effects of the thiol oxidants on enzyme responsiveness to activation by MNNG were prevented by dithiothreitol. These results suggest that the activation of particulate guanylate cyclase by nitric oxide or MNNG involves the oxidation of key enzyme sulfhydryl groups.

Braughler, J. M.

Biochemical Pharmacology 31(7):1239-1244, 1982.

From the Program in Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown.

SEPARATION AND DETECTION OF LIPOPROTEINS IN HUMAN SERUM BY USE OF SIZE-EXCLUSION LIQUID CHROMATOGRAPHY: A PRELIMINARY REPORT

This methodological paper presents a relatively mild and quite rapid procedure for separating serum lipoproteins for individual collection. In a first step, human serum components, including lipoproteins, can be rapidly separated by size-exclusion "high-performance" liquid chromatography. Then, lipoproteins in fractions of the eluate can be quantitated by conventional chemical and enzymatic methods. Alternatively, if lipoproteins in the serum are selectively prestained with diformazan dye, the column effluent can be monitored spectrophotometrically at 580 nm, so that only the lipoprotein components of serum are detected. Samples of purified low-density lipoproteins, so stained and analyzed, provide peak-area values that are proportional to their concentration as evaluated by chemical methods. With this technique, the various lipoprotein classes can be quickly separated and their concentration estimated. Overall, these techniques seem to have potential for development into analytical and clinical procedures.

Busbee, D. L. et al.

Clinical Chemistry 27(12):2052-2058, 1981.

Other support: American Cancer Society, Robert A. Welch Foundation and a North Texas State University Faculty Research Grant.

From the Departments of Biological Sciences and Chemistry, North Texas State University, Denton, and the Department of Biochemistry, Texas College of Osteopathic Medicine, Ft. Worth.

A NEW SUBCUTANEOUSLY-IMPLANTABLE RESERVOIR FOR SUSTAINED RELEASE OF NICOTINE IN THE RAT

Nicotine has become a widely studied drug for its pharmacological and toxicological effects. In previous animal studies, forced-administration methods for nicotine included parenteral injection, administration by inhalation of cigarette smoke, solubili-

zation in drinking water, and injection in single doses or chronically via Alzet minipump into the ventricles of the brain, among others. This paper now reports the successful development of a subcutaneously-implantable reservoir for the sustained release of nicotine. The device, dubbed INR for Implantable Nicotine Reservoir, is a small glass cup sealed with Silastic® polymer. It releases 3.4 mg of nicotine per 24 hours. When implanted into moderately-sized female Sprague-Dawley rats it produces blood nicotine levels of 400-500 ng/ml which remain relatively stable over at least 18 days. INRs are nontoxic, reproducible, inexpensive, and adaptable for pharmacological and toxicological studies in rats and other small animals.

Erickson, C. K. et al.

Pharmacology Biochemistry & Behavior 17:183-185, 1982.

From Drug Dynamics Institute, College of Pharmacy, The University of Texas, Austin.

DETERMINATION OF THE PRIMARY METABOLITE OF CENTRAL NERVOUS SYSTEM NOREPINEPHRINE, 3-METHOXY-4-HYDROXYPHENETHYLENEGLYCOL, IN MOUSE BRAIN AND BRAIN PERFUSATE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

The rate of production of 3-methoxy-4-hydroxyphenethyleneglycol (MHPG), the principal metabolite of norepinephrine in mamalian brain, has been proposed to be an indicator of the rate of norepinephrine turnover. For this reason, new methods of MHPG isolation and detection are important in furthering man's knowledge of the role of norepinephrine in normal and dysfunctional mamalian brain. In the present methodology paper, assays are described for the determination of picomole levels of MHPG in mouse brain and in the perfusate of an intact mouse brain. High-performance liquid chromatography with electrochemical detection yielded a MHPG detection limit of 0.37 pmol. This technique offers a sensitive and inexpensive alternative to gas chromatography with mass spectrometry, and can be used in conjunction with brain catecholamine determinations.

Towell, J. F. and *Erwin, V. G.*

Journal of Chromatography 223:295-303, 1981.

Other support: U. S. Public Health Service.

From the School of Pharmacy, University of Colorado, Boulder.

PRIMARY STRUCTURE OF THE SIGNAL PEPTIDE OF TROPOELASTIN b

Elastin is a major structural component of connective tissues. Its soluble precursor, tropoelastin, is extractable in organic solvents and possesses an extensive clustering of nonpolar amino acid residues in the immediate NH_2-terminal region. Since many proteins secreted from eukaryotic cells are initially synthesized with signal peptides, tropoelastin was examined to see if it did in fact contain a signal peptide. Leader sequences, or signal peptides, are relatively short hydrophobic NH_2-terminal exten-

sions, which are thought to play a role in vectorial transport of the nascent polypeptide. Therefore, the possibility that the initial tropoelastin translation product possesses a short signal peptide was examined in a cellfree translation system. In this study, total RNA, isolated from aortae of 1-day-old chicks, was translated in an mRNA-dependent reticulocyte lysate translation assay. The translation products were then immunoprecipitated and subjected to automated radio-sequencing. Comparison of the NH_2-terminal sequence of tropoelastin b synthesized in the cell-free system versus that synthesized in organ culture demonstrated the presence of a signal peptide 24 amino acid residues in length. The signal peptide sequence is as follows: Met-Arg-Gln-Ala-Ala-Ala-Pro-Leu-Leu-Pro-Gly-Val-Leu-Leu-Leu-Phe-Ser-Ile-Leu-Pro-Ala-Ser-Gln-Gln. The preponderance of hydrophobic amino acid residues as well as the polar residues adjacent to the initiator methionine and the carboxyl termini found in the signal peptide is similar to that reported for other secreted proteins.

Karr, S. R. and *Foster, J. A.*

The Journal of Biological Chemistry 256(12):5946-5949, 1981.

Other support: National Institutes of Health.

From the Department of Biochemistry, University of Georgia, Athens.

ABSENCE OF SEASONAL VARIATION IN ANTIPYRINE METABOLISM

Aryl hydrocarbon hydroxylase (AHH), the monooxygenase system(s) that metabolizes benzo(α)pyrene to fluorescent phenols, has been shown to exhibit a strong seasonal variation. The *in vivo* metabolism of antipyrine, which is also catalyzed by microsomal cytochrome P-450-dependent monooxygenases, has been reported to be correlated with AHH inducibility in human lymphocytes. In the study presented here, an attempt was made to determine whether antipyrine metabolism, like AHH metabolism, shows seasonal changes. To do this, antipyrine half-life (t1/2) was measured in 10 nonsmokers and eight smokers in the summer and the winter, the two times of the year that correspond to the high and low peaks of inducible AHH activity as measured in lymphocytes. Results showed that the mean antipyrine t1/2 determined in all 18 subjects in summer was almost identical to that found in winter ($x \pm SEM = 10.90 \pm 0.65$ and 10.96 ± 0.78 hr). AHH activity in cultured human lymphocytes from the nonsmoking subjects was determined in control and 3-methylcholanthrene-induced cells to obtain inducibility ratios of 4.2 ± 0.56 (SEM) in the summer and 1.4 ± 0.14 (SEM) in the winter. These results indicate that the seasonal variation in AHH inducibility in human lymphocytes is not reflected by a corresponding seasonal variation in antipyrine metabolism *in vivo*.

Paigen, B., Ward, E., Steenland, K., Bolanowska, W., *Gessner, T.*, Chang, R. L., Wood, A. W., and Conney, A. H.

Clinical Pharmacology & Therapeutics 31(2):144-150, 1982.

Other support: National Cancer Institute.

From the Departments of Molecular Biology and Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo, and the Department of Biochemistry and Drug Metabolism, Hoffmann-LaRoche Inc., Nutley, NJ.

ENZYMATIC PROPERTIES OF HUMAN GLUCURONYLTRANSFERASE AND A SENSITIVE METHOD FOR ITS ASSAY IN A STABLE B LYMPHOCYTE CELL LINE

UDP-glucuronyltransferases are membrane-bound enzymes located chiefly in the endoplasmic reticulum of cells and are responsible for the conjugation of endogenous and xenobiotic substances containing hydroxyl, amino, thiol, or carboxylic functional groups. Although there is much interest in human glucuronyltransferases, extensive studies of them have been hampered by difficulties associated with procurement of sufficient quantities of fresh human cells. This report, however, demonstrates that a prolific stable cell line, such as SN1006, capable of yielding billions of cells could be used for such studies. Homogenates of SN1006 cells have been studied. A sensitive assay procedure for lymphocyte glucuronyltransferase was developed utilizing radioactive testosterone as the acceptor substance and TLC for separation of the metabolite. The method is capable of detecting picomolar quantities of the product. The enzyme activity exhibited a broad pH optimum, and was subject to activation by the detergent Lubrol WX and Mn^{++} ions. The activity conformed to the Michaelis-Menten kinetics giving apparent K_m values of 0.8 mM and 11 μM, for UDPGA and testosterone, respectively. 4-Methylumbelliferone, α-naphthol and *P*-nitrophenol behaved as competitive inhibitors of testosterone glucuronidation. The results presented in this paper indicate that the method could be used for genetic studies of human lymphocyte glucuronyltransferase, and that the enzyme is of consequence in detoxication of exogenous as well as endogenous substrates.

Li, H. C., Porter, N. and *Gessner, T.*

Enzyme 28:54-65, 1982.

Other support: U. S. Public Health Service and the New York State Department of Health.

From the Department of Experimental Therapeutics and Grace Cancer Drug Center, Roswell Park Memorial Institute, Buffalo.

SUBSTRATE SPECIFICITY OF HUMAN UDP-GLUCURONYLTRANSFERASE IN CULTURED LYMPHOCYTES

Human lymphocytes possess glucuronyltransferase activity with a broad range of substrate specificity. For the paper presented here, catalytic properties of lymphocyte glucuronyltransferase were studied, and activity for the following substances was documented: testosterone, estradiol, phenolphthalein, α-naphthol, 4-methylumbelliferone and *p*-nitrophenol. As has been noted before, α-naphthol is regarded as a model aglycone for the form of glucuronyltransferase which catalyses glucuronidation of the less bulky nonsteroidal substrates, and testosterone as that for the steroidal aglycones. The results show that human lymphocytes possess glucuronyltransferase activity for both types of aglycone. Within limitations of work with crude homogenates and 60 minute incubations, no difference could be seen in apparent K_m values of UDPGA

when the various aglycone substrates were tested. Furthermore, competitive inhibition of testosterone UDP-glucuronyltransferase was observed when 4-methylumbelliferone, α-naphthol and *p*-nitrophenol were used as the inhibitors. These data demonstrate the similarity of the enzyme(s) accepting the various substrates.

Li, H. C., Porter, N., Holmes, G., and *Gessner, T.*

Xenobiotica 11(10):647-654, 1981.

Other support: New York State Department of Health.

From the Department of Experimental Therapeutics and Grace Cancer Drug Center, Roswell Park Memorial Institute, Buffalo.

STUDIES ON THE DEPOSITION AND DISTRIBUTION OF CATECHOL FROM WHOLE CIGARETTE SMOKE IN BCF1/CUM MICE

The studies reported here were performed to determine the deposition, distribution, and clearance of catechol in cigarette smoke using defined smoke exposure conditions and a well-characterized mouse strain (BC3F1/Cum). The presence of [^{3}H]catechol in the smoke was verified by silica gel chromatography, high-performance liquid chromatography, and gas chromatography/mass spectrometry. Mice were exposed to 10%(v/v)2R1 cigarette smoke on the Walton Horizontal Smoking Machine under standard conditions of 35 ml puff volume, 2 sec/puff, 10 puffs/cigarette. The deposition and distribution of inhaled catechol were determined in all internal tissues, urine, and feces. Data showed that clearance was occurring during the 10-min smoke exposure period. Immediately after exposure, over 50% of the radioactivity was found in the blood, with 10% found in the lung, and approximately 12% in the respiratory tract. Over 91% of the inhaled radioactivity was found in the urine 120 min after exposure. Less than 0.5% of the total dose was found in the lung at this time. In summary, these determinations show that catechol in smoke is rapidly absorbed, redistributed and excreted from mice exposed to whole cigarette smoke.

Hwang, K. K., Sonko, O., Dansie, D. R., Kouri, R. E., and Henry, C. J. *(Microbiological Associates)*

Toxicology and Applied Pharmacology 64(3):405-414, 1982.

From the Department of Experimental Oncology, Microbiological Associates, Bethesda, MD.

EFFECTS OF NICOTINE ON UTERINE BLOOD FLOW AND INTRAUTERINE OXYGEN TENSION IN THE RAT

Since it was known that normal conceptus development requires optimal levels of oxygen, it seemed reasonable to determine whether nicotine-induced reduction in uterine blood flow concomitantly decreases the concentration of oxygen within the uterine lumen. In the study presented here, it was seen that subcutaneous injection of

nicotine (0.5 or 5 mg/kg body wt) resulted in a marked and prolonged reduction in uterine blood flow and intrauterine oxygen tension in pseudopregnant rats (Day 4). By 10 minutes after nicotine administration (5 mg/kg) uterine perfusion was reduced by 40%, remained suppressed for 90 minutes and returned to the pre-treatment level by 120 minutes. Rats receiving the 0.5 mg nicotine/kg also showed a marked reduction in uterine blood flow, although the response was slower in onset and longer in duration. Nicotine (5 mg/kg), also resulted in a sustained decrease in intrauterine oxygen tension from a control value of 48.9±3.6 to 22.2±2.6 mmHg at 45-60 minutes and 21.7±1.5 mmHg at 60-90 minutes. The frequency and amplitude of fluctuations in intrauterine oxygen tension were still reduced by 90 minutes after treatment. Therefore, the results of this study indicate that nicotine, in amounts sufficient to suppress embryonic growth, reduces uterine blood flow and produces a marked and sustained decrease in oxygen tension within the uterine lumen. The time-course of the reduction in intrauterine oxygen availability parallels that of the nicotine-induced decrease in uterine blood flow.

Hammer, R. E., Goldman, H. and *Mitchell, J. A.*

Journal of Reproduction and Fertility 63:163-168, 1981.

From the Departments of Anatomy and Pharmacology, Wayne State University School of Medicine, Detroit.

SYNTHESIS OF NON-K-REGION *ORTHO*-QUINONES OF POLYCYCLIC AROMATIC HYDROCARBONS FROM CYCLIC KETONES

In this methodological paper, non-K-region *o*-quinones of polycyclic aromatic hydrocarbons are prepared easily by heating a solution of a tetrahydrodiol with 12 equiv. 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in dioxane (reflux, 24 hrs). DDQ not only oxidizes the 1,2-diol moiety α-diketone but also introduces the olefinic double bond of the *o*-quinone. When this one-step conversion was first discovered, the surprisingly facile generation of the *o*-quinone was attributed to the ease of double bond formation at the bay-region as described for several tetrahydro arenes. However, present results indicate that the reaction is not restricted to the synthesis of non-bay-region *o*-quinones but is also applicable to the preparation of bay-region *o*-quinones. Thus, this method is both simpler and more general than the recently published synthesis of non-K-region *o*-quinones. Overall, the synthetic approach presented here constitutes a new nethod for the preparation of non-K-region *o*-quinones that seems to be generally applicable.

Platt, K. L and *Oesch, F.*

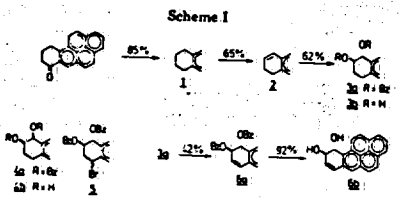
Tetrahedron Letters 23(2):163-166, 1982.

From the Department of Toxicology, Institute of Pharmacology, University of Mainz, Mainz, Federal Republic of Germany.

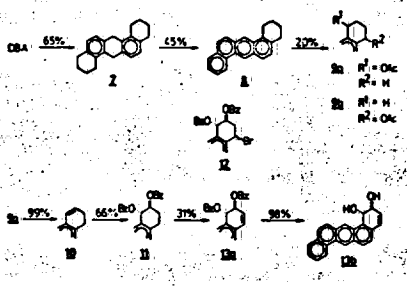
IMPROVED SYNTHESIS OF (±)-TRANS-9-10-DIHYDROXY-9-10-DIHYDRO-BENZO[α]-PYRENE AND OF (±)-TRANS-1-2,-DIHYDROXY-1,2-DIHYDRO-DIBENZ[*a,h*]ANTHRACENE

Non-K-region dihydrodiols of polycyclic aromatic hydrocarbons (PAH) play an important role in the metabolism of PAH. They are precursors of dihydrodiol epoxides, some of which are considered ultimate carcinogenic metabolites of PAH. While the non-K-region dihydrodiols with the olefinic bond in the bay region have been intensively studied, the biological role of the non-K-region dihydrodiols of benzo(α)pyrene and dibenz[*a,h*] anthracene (6b and 13b), where the olefinic bond is not of the bay region, is less well known. In the present study, improved synthetic pathways for 6b and 13b were devised that resulted in the schemes seen below: a six-step synthesis of 6b (Scheme I) and eight-step synthesis of 13b (Scheme II).

Scheme I



Scheme II



Since the little interest in the dihydrodiols 6b and 13b stems partly from the fact that their syntheses described in the literature are more difficult than those of the better studied isomers, it is hoped that these new improved syntheses may help in furthering study of the biological roles of 6b and 13b.

Oesch, F. et al.

The Journal of Organic Chemistry 47:568-571, 1982.

From the Institute of Pharmacology, Section of Biochemical Pharmacology, University of Mainz, Mainz, Federal Republic of Germany.

K-REGION *TRANS*-DIHYDRODIOLS OF POLYCYCLIC ARENES; AN EFFICIENT AND CONVENIENT PREPARATION FROM *O*-QUINONES OR *O*-DIPHENOLS BY REDUCTION WITH SODIUM BOROHYDRIDE IN THE PRESENCE OF OXYGEN

K-region *trans*-dihydrodiols are important metabolites of polycyclic aromatic hydrocarbons. They are produced by epoxide hydrolase-mediated hydration of the primarily formed arene oxides. The K-region *trans*-dihydrodiols are usually synthesized by stereoselective reduction of the corresponding *o*-quinones with complex metal hydrides such as lithium aluminum hydride. When first attempts were made to use sodium or potassium borohydride in methanol or ethanol for the reduction of K-region *o*-quinones, they were not very successful. Also, the use of different reaction times and temperatures, different ratios of the reactants, and different solvents did not improve

the results substantially. However, it was finally found that polycyclic *o*-quinones can be conveniently reduced to the corresponding *trans*-dihydrodiols by carrying out the reduction with borohydrides in the presence of oxygen. The role of oxygen in the reduction may be rationalized by a mechanism presented in this paper. As a result of the reduction-oxidation cycle that takes place when oxygen is available, pure *trans*-dihydrodiol accumulates so that it can be isolated in high yield. This new method of reducing K-region *o*-quinones (and also the corresponding *o*-diphenols and their acetates) derived from polycyclic arenes represents a significant improvement of the synthesis of K-region *trans*-dihydrodiols.

Platt, K. L. and *Oesch, F.*

SYNTHESIS: International Journal of Methods in Synthetic Organic Chemistry No. 6:459-462, June 1982.

From the Institute of Pharmacology, Section on Biochemical Pharmacology, University of Mainz, Mainz, Federal Republic of Germany.

ANALYSIS OF NICOTINE AND COTININE IN TISSUES BY CAPILLARY GAS CHROMATOGRAPHY AND GAS CHROMATOGRAPHY—MASS SPECTROMETRY

In this methodological paper, the development and application of methods to quantitate low levels of nicotine and cotinine in tissue samples are described. Analyses were performed by capillary column gas chromatography with a specific nitrogen-phosphorus detector and by gas chromatography-mass spectrometry. With close structural analogs for internal standards, high quantitative accuracy and precision were demonstrated for the range of 5-1000 ng per g of tissue. The sensitivity limit was 2-3 ng/g for both compounds. The main advantage of these techniques compared to previously published methods is increased selectivity; the other methods were developed for analysis of biological fluids and are not readily adaptable to more complex biological matrices such as tissue homogenates. With the newly developed techniques, it was possible to perform a pharmacokinetic study of nicotine and cotinine in mouse liver following a single intraperitoneal injection of nicotine.

Thompson, J. A., Ho, M-S. and *Petersen, O. R.*

Journal of Chromatography 231:53-62, 1982.

From the School of Pharmacology, University of Colorado, Boulder.

RELATIONSHIPS BETWEEN CHEMICAL STRUCTURE AND CHOLINERGIC ACTIVITIES OF FURMETHIDES AND POTENTIATION OF THESE ACTIVITIES BY PHYSOSTIGMINE

The furmethide compounds–furfuryltrimethylammonium (furmethide; FT), 5-methylfurmethide (5-MFT), 5-hydroxymethylfurmethide (5-HMFT) and 5-chloromethylfurmethide (5-ClMFT)–are potent and stable muscarinic agents which are being used in pharmacological investigations where muscarinic selectivity is required. For the studies reported here, the cholinergic activities of the four furmethides were compared on a superfused guinea pig ileum preparation. Results showed that all four components were less potent but equally as active as acetylcholine (ACh). The ED50s

of these compounds decrease in the following order: 5-C1MFT > 5-HMFT > FT > 5-MFT. The electron densities around the furan-oxygen atoms of these compounds decrease in the same order. A careful analysis of the effects of these agents on the guinea pig ileum revealed that the furmethides display two separate actions: (1) direct interaction at the muscarinic receptor to elicit a response, and (2) release of ACh from the presynaptic nerve terminal. In the presence of physostigmine, dose response curves of these compounds were shifted to the left. There was approximately a two-fold shift in the ED50 values for the furmethide. Like muscarine, these compounds were not hydrolyzed by cholinesterase (ChE). At least part of the noted furmethides response was due to release of ACh, because the responses were increased by ChE inhibition.

Chaturvedi, A. K., Rowell, P. P., and *Sastry, B. V. R.*

Pharmacological Research Communications 13(9):829-845, 1981.

Other support: National Institute of Child Health and Human Development.

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

CHANGES IN MICROSOMAL MEMBRANE MICROVISCOSITY AND PHOSPHOLIPID METHYLTRANSFERASES DURING RAT LIVER REGENERATION

Regenerating liver is a good example of a controlled growing tissue. During liver growth, newly synthesized molecules of drug-metabolizing enzymes like cytochrome P450 are incorporated into the lipid matrix of endoplasmic reticulum (ER). There is a morphological association of ribosomal protein synthesis with ER membranes. Recent research has shown that decreased membrane microviscosity facilitates incorporation of the enzyme-protein molecules into the phospholipid matrix of ER. The microviscosity of membranes is altered by: (1) changed ratios of phospholipid to cholesterol, (2) unsaturation of fatty acids in phospholipids, and (3) methylation of phospholipids. In a related study, microviscosity and S-adenosyl-L-methionine (SAM) mediated methylation of phosphatidylethanolamine to phosphatidyl-N-methylethanolamine (PME) and phosphatidylcholine (PC) were measured using microsomal membranes of regenerating rat livers at 6-96 hrs after partial hepatectomy. In the methylated phospholipids, the proportion of PME increased by 3-9% at 1 μM SAM, and, at 200 μM SAM, the proportion of PC decreased by about 5-10% at 12-24 hrs. Two phase transitions were observed with microsomal membranes between 20 and 40°C. In synthetic liposomes containing PE, PME and PC, microviscosity decreased when the proportion of PME increased or the proportion of PC decreased. Therefore, alterations in phospholipid methyltransferases and consequent changes in membrane phospholipid methylation may contribute to increased membrane fluidity during cell proliferation and incorporation of drug-metabolizing enzymes into ER.

Jaiswal, R. K., *Sastry, B. V. R.* and Landon, E. J.

Pharmacology 24:355-365, 1982.

Other support: U. S. Public Health Service.

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

ENHANCEMENT OF THE RESPONSIVENESS OF THE RAT DIAPHRAGM BY L-METHIONINE AND PHOSPHOLIPID METHYLATION AND THEIR RELATIONSHIP TO AGING

The contractions of hemidiaphragms of Fischer 344 rats of age 2 to 33 months were measured after electrical stimulation of the phrenic nerve or the muscle *in vitro*. Earlier work has shown that S-adenosyl-L-methionine (SAM)-mediated methylation of membrane phospholipids increases membrane fluidity and responsiveness of the muscle, and that an increase in the intracellular levels of S-adenosyl-L-homocysteine (SAH) inhibits phospholipid methylation. In the study presented here, the intracellular levels of SAM were increased by incubating the hemidiaphragm in L-methionine, L-homocysteine thiolactone, adenosine and erythro-9-(2-hydroxy-3-nonyl) adenine, an inhibitor of adenosine deaminase. The following results were obtained: (1) Microsomes from hemidiaphragm contained phospholipid methyltransferases, (2) L-methionine increased muscle tension developed by electrical stimulation of the muscle or the nerve. This increase in the tension is dependent upon the concentration of L-methionine. (3) Labeled methyl groups were incorporated from labeled L-methionine into phospholipids of the hemidiaphragm. (4) Presence of adenosine, L-homocysteine thiolactone and erythro-9-(2-hydroxy-3-nonyl)adenine inhibited the effect of L-methionine to increase muscle tension and incorporation of methyl groups into phospholipids. (5) Muscle tension developed by electrical stimulation of the phrenic nerve or the muscle decreased with increasing age, and (6) The methionine effect was erratic or insignificant in hemidiaphragms of old rats (>15 months). These observations indicate that increasing the cellular formation of SAM increases phospholipid methylation and the contraction heights of the hemidiaphragm upon electrical stimulation. Both of these effects are inhibited by increasing levels of SAH. They also indicate that SAM-mediated membrane phospholipid methylation and fine regulation of membrane fluidity in the diaphragm may become defective with advancing age, and may contribute partly to the functional deficits of the diaphragm.

Sastry, B. V. R., Owens, L. K. and Janson, V. E.

The Journal of Pharmacology and Experimental Therapeutics 221(3):629-636, 1982.

Other support: U.S. Public Health Service, National Institute of Aging, and the National Institute of Child Health and Human Development.

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

REGULATION OF ACETYLCHOLINE RELEASE IN THE MOUSE CEREBRUM BY METHIONINE ENKEPHALIN AND SUBSTANCE P

Methionine enkephalin (MEK) and Substance P (SP) may regulate acetylcholine (ACh) release in the cerebrum. In the present attempt to study this process, mouse cerebral slices were incubated in a modified Krebs bicarbonate buffer containing (methyl-^{3}H) choline chloride. The slices were filtered, washed and transferred to a microbath set up for superfusion. ACh release was followed by the efflux of labeled

ACh and choline as a function of time. The release of opioid peptides was measured by specific radioimmunoassays. The rate of ^{3}H-ACh release increased initially for the first five min., reached a peak, and then declined exponentially (half-time, 35 min.). Electrical field stimulation of the slices during the exponential phase caused a significant increase in ACh release for five min. Absence of exogeneous Ca^{++} in the perfusion field depressed both spontaneous and evoked release of ACh. When the effects of long acting enkephalin (D-ala-enkephalinamide, DALA) and SP on ACh release were studied, DALA (34 nM) decreased the release of ACh (65%) as well as Ca^{++} uptake (48%). SP(6×10^{-7} M) increased ACh release (35%) and increased Ca^{++} uptake (154%). These observations indicate that enkephalins may exert a negative feedback control of ACh release from cerebral slices by inhibiting Ca^{++} uptake by the slices. Similarly, SP may exert a positive feedback on the ACh release by enhancing the uptake of Ca^{++} by cerebral slices.

Sastry, B. V. R. and Tayeb, O. S.

In: Dhawan, B. N. (ed.): *Current Status of Centrally Acting Peptides*, New York: Pergamon Press, 1982, pp. 165-172. (also in: *Advances in the Biosciences:* 165-172, 1982.)

Other support: U.S. Public Health Service.

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN.

ION BEAM TRITIUM LABELING OF PROTEINS AND PEPTIDES

An advanced technique for tritiating proteins that yields high specific radioactivities without causing significant changes in biological activities is presented here. In this method, a carefully controlled particle beam composed of T_3^+ and T_2^+ ions and fast T_2 molecules is accelerated into a sample target within a vacuum chamber. This beam method has been used to tritiate ribonuclease A, porcine pancreatic elastase, thermolysin, soybean trypsin inhibitor, α_1-protease inhibitor, and the peptide aldehydes leupeptin and antipain. After removal of all readily exchangeable tritium, the products were obtained in 32-83% yields with specific radioactivities of 18-856 Ci/mol. The products were carefully characterized, shown to be chemically pure, and to have complete biological activity. Simple tritium hydrogen exchange accounts for at least 82% of the reaction pathway with proteins and for 100% of the reaction with the peptide aldehydes. This method is practical and economical on a reasonable scale, producing high specific tritium incorporation without significant chemical decomposition or structural change to even fragile, high molecular weight materials.

Bush, G. A. *et al. (Travis, J.)*

The Journal of Biological Chemistry 256(23):12213-12221, 1981.

Other support: National Institutes of Health.

From the School of Chemistry, Georgia Institute of Technology, Atlanta.

ISOLATION AND PROPERTIES OF HUMAN NEUTROPHIL MYELOPEROXIDASE

Purification of human leukocyte myeloperoxidase, as described in this paper, is a simple three-step procedure involving dialysis of a granule extract against low-salt buffer, Sephadex G-75 chromatography, and carboxymethylcellulose chromatography. The final product of this purification is homogeneous when examined by acid polyacrylamide gel electrophoresis and sedimentation equilibrium ultracentrifugation. The molecular weight that was determined here by the latter procedure was 118,000. With or without reduction of the protein by 2-mercaptoethanol, subunits were formed which migrated as a single band after sodium dodecyl sulfate gel electrophoresis. The molecular weight of the apparently identical subunits was 59,000 with reduction, and 42,000 without reduction. Other general properties of human leukocyte myeloperoxidase, including amino acid composition, amino terminal sequence analysis, and absorption spectra, are also reported. Myeloperoxidase, in the presence of hydrogen peroxide and chloride ion, and no other substrate, autoinactivates. After completion of the inactivation reaction, several oxidizable amino acids in the enzyme are modified, and the absorption peak at 430 nm disappears. The presence of substrate of the myeloperoxidase system (α-1-proteinase inhibitor), or of high concentration of chloride ion, completely protects the enzyme from autoinactivation.

Matheson, N. R., Wong, P. S. and *Travis, J.*

Biochemistry 20(2):325-330, 1981.

Other support: National Institutes of Health.

From the Department of Biochemistry, University of Georgia, Athens.

INTERACTION OF HUMAN α_1-PROTEINASE INHIBITOR WITH NEUTROPHIL MYELOPEROXIDASE

In earlier studies, it has been shown that α-1-proteinase inhibitor (α-1-PI) can be inactivated by myeloperoxidase in the presence of hydrogen peroxide and chloride ion. Since myeloperoxidase may be readily released from neutrophils, its oxidative inactivation of α-1-PI may indirectly result in enhancement of proteolytic destruction of lung tissue, even in individuals with genetically normal levels of inhibition. The aim of this investigation, therefore, was to further investigate the parameters of the myeloperoxide-mediated oxidative inactivation of α-1-PI. These studies, as reported here, demonstrate that there is a direct dependence on the concentration of α-1-PI which becomes saturating at a concentration of about 4.5 μM. There is also a dependence on the concentration of H_2O_2 to 95 μM, after which increasing concentrations become increasingly inhibitory. Chloride ion is required for myeloperoxidase oxidative action, but the cations Na^+, NH_4^+, or K^+ have little effect. There is a very sharp pH optimum at pH 6.2, under physiological NaCl concentration, and approximately half the rate of inactivation of α-1-PI occurs at pH 5.9 or 6.5. Sodium dodecyl sulfate gel electrophoresis indicates that α-1-PI oxidized by either myeloperoxidase or N-chlorosuccinimide is not different in size from native α-1-PI. When oxidized α-1-PI is incubated with porcine elastase, the α-1-PI is converted to a modified form of lower molecular weight. Amino acid sequence analysis confirms these results in that the sequence is different from that of native α-1-PI. Overall, from this data and other observations reported here, it is apparent that oxidative processes directly affect the inhibitory activity of α-1-PI modification of the reactive-site methionine of this protein.

Matheson, N. R., Wong P. S., Schuyler, M., and *Travis, J.*

Biochemistry 20(2):331-336, 1981.

Other support: National Institutes of Health.

From the Department of Biochemistry, University of Georgia, Athens.

INACTIVATION OF HUMAN PLASMA α_1-PROTEINASE INHIBITOR BY A METALLOPROTEINASE FROM *SERRATIA MARCESCENS*

This study was undertaken to investigate the interaction of human plasma α_1-proteinase inhibitor with a metalloproteinase isolated from *Serratia marcescens*. Results presented here show that the interaction of α_1-proteinase inhibitor with the *Serratia* proteinase caused a rapid decrease in inhibitory activity towards trypsin which was both concentration- and time-dependent. To determine whether the inactivation had occurred by limited proteolysis, proteinase-α_1-proteinase inhibitor incubation mixtures were denatured and analyzed by SDS-polyacrylamide gel electrophoresis. These results show that the proteinase had rapidly converted α_1-proteinase inhibitor into an inactive form of lower molecular weight (48,000 for the modified form vs. 52,000 for the native inhibitor). Amino terminal sequence analysis indicated that the interaction of the inhibitor and enzyme was at the reactive site of the inhibitor, with peptide-bond cleavage resulting in the inactivation. This process may be important in necrotic processes occurring during bacterial infiltration of host tissues.

Virca, G. D., Lyerly, D., Kreger, A., and *Travis, J.*

Biochimica et Biophysica Acta 704:267-271, 1982.

Other support: National Institutes of Health.

From the Department of Biochemistry, University of Georgia, Athens, and the Department of Microbiology and Immunology, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC.

VI. *Immunology and Adaptive Mechanisms*

CHEMOTACTIC ACTIVITY GENERATED FROM THE FIFTH COMPONENT OF COMPLEMENT BY PLASMA KALLIKREIN OF THE RABBIT

Kallikrein purified from rabbit plasma has been shown in the study presented here to generate chemotactic activity for rabbit neutrophils from the rabbit's fifth component of complement (C5). The effect on C5 appeared to be due to kallikrein itself and not to a contaminating enzyme, because it could be inhibited by anti-kallikrein IgG or by soybean trypsin inhibitor to the same extent that kinin generation by the same

kallikrein preparation was inhibited by these agents. The chemotactic response was consistent with the generation of a C5a-like peptide from C5, because the effect could be partially inhibited by carboxypeptidase N and was related to the generation of a small (~14,000 mol wt) fragment of C5. In contrast, no chemotactic activity could be demonstrated when the zymogen prekallikrein was tested with C5 under identical conditions. Chemotactic activity was also generated when rabbit C5 was incubated with the 80,000-M, form of activated Hageman factor, trypsin or EAC423. No chemotactic activity was produced when C5 was absent from the incubation mixtures or when intact C5 alone was assayed. In sum, these results suggest the existence of a novel interaction between the Hageman factor and complement systems which may have biological relevance.

Wiggins, R. C., Giclas, P. C. and Henson, P. M. *(Cochrane, C. G.)*

Journal of Experimental Medicine 133:1391-1404, 1981.

Other support: National Institutes of Health and the Office of Naval Research.

From the Department of Immunopathology, Research Institute of Scripps Clinic, La Jolla, CA.; and the Department of Pediatrics, National Jewish Hospital, and Departments of Medicine and Pathology, University of Colorado Medical School, Denver.

GUINEA PIG HAGEMAN FACTOR AS A VASCULAR PERMEABILITY ENHANCEMENT FACTOR

In this ongoing attempt to ascertain the biological role of the contact (Hageman factor) system, Hageman factor was purified from guinea pig plasma by successive column chromatography, and an active Hageman factor, β-HFa, was prepared for study. The guinea pig Hageman factor appeared homogeneous as a single-chain protein on polyacrylamide gels in the presence of sodium dodecyl sulfate and β-mercaptoethanol. Amino acid composition of the guinea pig Hageman factor was similar to that reported for human, bovine, and rabbit Hageman factors. The purified guinea pig Hageman factor, as well as guinea pig plasma, showed strong clotting time correction activity in Hageman-factor-deficient human plasma. The activity could be blocked by the IgG fraction of antiserums against guinea pig Hageman factor raised in rabbits or a goat. The concentration of Hageman factor in guinea pig plasma was determined to be 120 μg/ml by quantitative radial immunodiffusion assay. When β-HFa, the 28,000-dalton active form of Hageman factor, was prepared from guinea pig Hageman factor by treatment with plasma kallikrein, β-HFa caused an increase in vascular permeability when injected into guinea pig skin at concentrations as low as 3×10^{-10} M. This increased permeability was short-lasting, and the permeability enhancement activity of β-HFa was inhibited by pretreatment of β-HFa with diisoprophylfluorophosphate. According to the authors, it may be concluded, therefore, that active Hageman factor in the interstitial space of guinea pigs acts as a vascular permeability factor of far greater potency than bradykinin.

Yamamoto, T. and *Cochrane, C. G.*

American Journal of Pathology 105(2):164-173, 1981.

Other support: National Institutes of Health and the Office of Naval Research.

From the Department of Immunopathology, Research Institute of Scripps Clinic, La Jolla, CA.

MODULATION OF POKEWEED-MITOGEN-INDUCED IMMUNOGLOBULIN SECRETION BY HUMAN BRONCHOALVEOLAR CELLS

The effects of pulmonary alveolar macrophages (PAM) on immunoglobulin (Ig) secretion were investigated, using autologous peripheral blood lymphocytes as the indicator population and pokeweed mitogen (PWM) as a monocyte-dependent polyclonal B-cell activator. Bronchoalveolar cells (BAC) from seven nonsmoking subjects suppressed the response to PWM by unfractionated autologous peripheral blood mononuclear cells (MNL), whereas low concentrations of BAC partially reconstituted the response of monocyte-depleted MNL to PWM. Thus, it could be seen that BAC could modulate PWM-induced Ig secretion in different ways, depending on the presence or absence of monocytes in the mononuclear cell population. The suppressor activities of BAC were not abrogated by prior irradiation and were only partially reversed by the addition of indomethacin to the cultures. However, prior disruption of BAC completely abolished their suppressive functions. Suppression of PWM-induced Ig secretion is probably mediated by intact, radioresistant PAM.

Lawrence, E. C., Theodore, B. J. and Martin, R. R.

American Review of Respiratory Disease 126:248-252, 1982.

Other support: American Lung Association and the National Institutes of Health.

From the Rockwell-Keough Pulmonary Immunology Laboratory and the General Clinical Research Center of the Methodist Hospital, and the Department of Medicine, Baylor College of Medicine, Houston.

DEFECTIVE IMMUNOGLOBULIN SECRETION IN RESPONSE TO POKEWEED MITOGEN IN SARCOIDOSIS

Several previous studies of sarcoidosis have indicated a dichtomy between enhanced humoral immune functions clinically and defective *in vitro* B cell responsiveness, which suggested some disorder of immunoregulation. In the study presented here, it was found that polyclonal immunoglobulin (Ig) secretory response to pokeweed mitogen (PWM) — a plant lectin which requires the presence of both monocytes and T cells in order to trigger B cells — was defective as well. Specifically, *in vitro* immunoregulation of Ig secretion was studied in 21 patients with sarcoidosis. While peripheral blood mononuclear cells from normal individuals responded to PWM with a 10-fold or greater increment in Ig-secreting cells, cells from sarcoid patients failed to respond to PWM at any concentration employed. More monocytes were found in sarcoid mononuclear cell preparations (44.8±2.0% vs 30.4±1.4% in normal donors), but removal of monocytes improved the response to PWM in only four patients. Mononuclear cells from seven of 19 patients suppressed Ig secretion in co-cultures with normal donor cells. Patients exhibiting excessive suppressor cell function were older, with longer standing and less clinically active disease than non-suppressing patients. Monocyte removal reversed the suppression in only four of the suppressor patients, but excessive suppressor monocyte function was later demonstrated in two sarcoid patients whose cells initially did not suppress Ig secretion when cultured with normal cells. While the immunological defects in sarcoidosis may be complex, heterogenous and

dynamic, these data suggest that suppressor monocytes, when present in sarcoidosis, may have developed secondarily.

Lawrence, E. C. et al.

Clinical and Experimental Immunology 49:96-104, 1982.

Other support: American Lung Association and the National Institutes of Health.

From the Rockwell-Keough Pulmonary Immunology Laboratory and the General Clinical Research Center of The Methodist Hospital, and the Department of Medicine, Baylor College of Medicine, Houston.

NEUTRAL GLYCOSPHINGOLIPIDS OF HUMAN ACUTE LEUKEMIAS

A methodology was introduced in this study which combines the sensitivity of high performance liquid chromatography with the specificity of exo- and endoglycosidases to study the neutral glycosphingolipids present in the malignant cells of 10 patients with acute leukemia. Results showed that acute leukemia cells contain very little or none of the more complex neutral glycosphingolipids that are found in normal leukocytes or chronic leukemia cells. Lymphoblasts, in particular, are rich in neutral glycosphingolipids with only one or two carbohydrate units. The most significant finding of this study was that, in contrast to normal leukocytes and chronic leukemia cells which have a single predominant tetraosylceramide species, acute leukemia cells (9 out of 10 patients analyzed) were found to have significant amounts of both globo (GalNAcβ1 Y 3Galα1 Y 4Galβ1 Y 4Glcβ1 Y lceramide) and neolactotetraosylceramide (Galβ1 Y 4GlcNAcβ1 Y 3Galβ1 Y 4Glcα1 Y lceramide). These results indicate that the composition of neutral glycosphingolipids in acute leukemia cells differs significantly from that found in normal or chronic leukemia cells.

Lee, W. M. F., Westrick, M. A. and *Macher, B. A.*

The Journal of Biological Chemistry 257(17):10090-10095, 1982.

Other support: National Institutes of Health, National Cancer Institute, Leukemia Research Foundation, and Cancer Research Funds of the University of California.

From the Cancer Research Institute, University of California, San Francisco.

GLYCOSPHINGOLIPIDS OF NORMAL AND LEUKEMIC HUMAN LEUKOCYTES

Studies on neutral glycosphingolipids and gangliosides of normal and leukemic human leukocytes were reviewed for this presentation. It can be seen here that two methodological approaches have been used to determine the structure and distribution of glycosphingolipids among human leukocytes: (1) those that utilize chemical and enzymatic tools to determine the complete structure, and (2) those that rely on indirect assays of detection. The former methods allow one to assign a unique structure to each compound, but they have two disadvantages: (1) they require relatively large quantities of materials and (2) minor components may be lost during the process of preparing homogenous compounds for analysis. Indirect methods which have been used are sensitive and rapid, and allow one to compare easily several samples, but they have the

disadvantages of being indirect, with structures assigned on the basis of comparison with standards. Examined for this review were: (a) the glycosphingolipid composition of various leukocyte populations, (b) the differences in glycosphingolipids found among subsets of these cells, (c) the possible use of these compounds as markers of differentiation, and (d) the changes in glycosphingolipid composition that occur with leukemogenesis.

Macher, B. A., Lee, W. M. F. and Westrick, M. A.

Molecular and Cellular Biochemistry 47:81-95, 1982.

Other support: National Institutes of Health, National Cancer Institute, Leukemia Research Foundation, and Cancer Research Funds of the University of California.

From the Cancer Research Institute and Department of Pharmaceutical Chemistry, University of California, San Francisco.

NEURON-SPECIFIC ENOLASE AS AN IMMUNOCYTOCHEMICAL MARKER FOR THE DIFFUSE NEUROENDOCRINE SYSTEM IN HUMAN FETAL LUNG

Neuron-specific enolase (NSE) is an isoenzyme of the glycolytic enzyme enolase, which was originally considered to be restricted to neurons but has recently been shown to occur in some APUD cells. In this paper, the localization of NSE in the diffuse neuroendocrine system of human fetal lung is reported. Specifically, NSE-positive cells, singly or in groups, were demonstrated by antisera raised to human or rat NSE. Immunostained serial sections indicated that NSE-positive cells could also contain bombesin and /or 5HT-like immunoreactivity. At least three different cell types were identified containing (1) NSE, 5HT, and bombesin, (2) NSE and 5HT, and (3) NSE alone. After close consideration of the material presented here, it appears that NSE is a useful marker of the neuroendocrine system in the lung as well as in other tissues. Also, the lack of alternative simple and reliable techniques capable of identifying both cells and nerves means that the immunocytochemical localization of NSE is a valuable tool for the study of development, physiology, and pathology of this system.

Wharton, J., *Polak, J. M.*, Cole, G. A., Marangos, P. J., and Pearse, A. G. E.

The Journal of Histochemistry and Cytochemistry 29(12):1359-1364, 1981.

From the Department of Histochemistry, Royal Postgraduate Medical School, London, England, and the Clinical Psychobiology Branch, National Institutes of Health, Bethesda, MD.

IgE-DEPENDENT RELEASE OF LEUKOTRIENE C_4 FROM ALVEOLAR MACROPHAGES

Slow reacting substances (SRS) have been shown recently to be a family of peptidolipids called leukotrienes (LTC_4, LTD_4 and LTE_4) that are derived from arachidonic acid and are potent bronchoconstrictors *in vivo* and *in vitro*. In the study presented here, rat alveolar macrophages are shown to be the cells responsible for releasing the SRS. The ability of these macrophages to release SRS was tested initially by incubating cell suspensions for 20 min with 1 μmol of calcium ionophore A23187,

in the presence of 5×10^{-4}M L-cysteine. In subsequent experiments, cell suspensions were stimulated with purified mouse monoclonal anti-DNP (dinitrophenyl) IgE antibody and DNP-human serum albumin. Results of these experiments show that (1) rat alveolar macrophages release SRS when stimulated non-specifically by the calcium ionophore A23187 in the presence of L-cysteine, and (2) IgE antibody and appropriate antigen cause alveolar macrophages to release SRS leukotriene, LTC_4. This demonstration that rat alveolar macrophages release SRS by an IgE-dependent mechanism raises the possibility that IgE-dependent release of mediators by alveolar macrophages may have a role in asthma or other immunologically mediated lung diseases.

Rankin, J. A. *et al. (Reynolds, H. Y.)*

Nature 297:329-331, 1982.

Other support: National Institutes of Health and Hoffman La-Roche Inc.

From the Department of Medicine and Pharmacology, Yale University School of Medicine, New Haven, and the John B. Pierce Foundation, New Haven, CT.

MONOCLONAL ANTIBODY ANALYSIS OF COMPLEX BIOLOGICAL SYSTEMS: COMBINATION OF CELL HYBRIDIZATION AND IMMUNOADSORBENTS IN A NOVEL CASCADE PROCEDURE AND ITS APPLICATION TO THE MACROPHAGE CELL SURFACE

In this sophisticated methodological paper, a procedure is described which greatly simplifies the collection of monoclonal antibody (MAb) libraries directed toward individual components of complex biological systems. For the study reported here, removal of previously recognized antigens with immunoadsorbent columns was combined with cell hybridization in a cascade which restricts the immunizing stimulus to previously unrecognized antigens. Specifically, in this report a cascade procedure was explored in connection with the identification of further macrophage-specific antigens. Peritoneal exudate cell membranes were detergent solubilized, and the previously identified common leukocyte antigen and heat-stable antigen which are shared with peritoneal exudate cells and lymphocytes were removed with MAb^2 immunoadsorbents before immunization for the hybridization experiment. Removal of the antigens was confirmed by radioimmunoassay and by the serological response to immunization. Serum antibodies to specific antigens were also measured to compare the efficacy of this procedure to immunization with either whole cells or MAb-coated cells. Two previously unknown macrophage-specific antigens of 32,000 and 110,000 M, were identified here. According to the authors, the precedure can be extended by arranging further immunoadsorbent depletions and cell fusions in a cascade series and is readily applicable to the monoclonal antibody analyses of many other multicomponent biological complexes.

Springer, T. A.

The Journal of Biological Chemistry 256(8):3833-3839, 1981.

Other support: U. S. Public Health Service.

From the Department of Pathology, Harvard Medical School, Boston.

MAC-1, 2, 3 AND 4: MURINE MACROPHAGE DIFFERENTIATION ANTIGENS IDENTIFIED BY MONOCLONAL ANTIBODIES

The Kohler-Milstein myeloma hybrid technique, which can enable the isolation of a monoclonal antibody recognizing a single antigenic determinant from an initial highly complex antigen, has given great impetus to the analysis of cell surface complexity. In the paper presented here, work done in the author's laboratory using this technique for the identification and study of macrophage antigens is reviewed. Four antigens — Mac-1,2,3 and 4 — have been identified by the corresponding monoclonal antibodies, Ml/70, M3/31, M3/38, M3/84 and M3/37. These antigens all appear to be on the macrophage cell surface on the basis of fluorescent and ^{125}I-labeling. ^{35}S-methionine incorporation into the polypeptides by the adherent fraction of thioglycollate-induced peritoneal exudate cells also suggests these antigens are synthesized by macrophages. The four different antigens defined in these studies are present on macrophages, but not lymphocytes, demonstrating the distinctiveness of macrophage cell surface architecture. Currently, the expression of these antigens on macrophages induced by other means and in different anatomical locations is being investigated. The monoclonal antibodies are also being used as probes to inhibit a panel of macrophage functions. In this way, it should be possible to link the molecular structures described here with specific macrophage cell surface activities.

Springer, T. A.

In: Förster, O. (ed.): *Heterogeneity of mononuclear phagocytes*, New York: Academic Press, 1980, pp. 37-46.

Other support: U. S. Public Health Service.

From the Department of Pathology, Harvard Medical School, Boston.

A SHARED ALLOANTIGENIC DETERMINANT ON Ia ANTIGENS ENCODED BY THE I-A AND I-E SUBREGIONS: EVIDENCE FOR I REGION GENE DUPLICATION

It has been known for a while that the I region of the major histocompatibility complex contains genes that control immune response and immune suppression to certain antigens and different determinants, and studies on these genes have led to the definition of a number of I subregions. In the study presented here, two rat monoclonal antibodies (MAb), M5/114 and M7/81, which have a very unusual type of crossreactive specificity for murine I region products, are characterized. These MAb detect polymorphic determinants present on B cells and activated T lymphocytes from mice carrying the H-2^b, H-2^d, H-2^k, H-2^r, and H-2^s haplotypes but not from mice carrying the H-2^a or H-2^f haplotypes. Antigenic site number determinations showed that the positive haplotypes can be divided into two groups. Mice bearing the H-2^b, H-2^d, and H-2^s haplotypes express a high number (40,000 to 80,000) of antigenic sites per B lymphocyte, and MAb plus complement can lyse B cells from these mice. In contrast, mice bearing the H-2^k and H-2^r haplotypes express a low number of antigenic sites. Spleen cells from mice carrying the latter haplotypes are not lysed with MAb and complement. Genetic mapping demonstrated that high and low expression map to the I-A and I-E subregions, respectively. The MAb detect an Ia specificity on I-A^b, I-A^d, I-E^d, and I-E^k molecules. These observations were confirmed using several different experimental approaches, i.e., cytotoxicity, fluorescent staining, competitive inhibition of MAb

binding, and 2-dimensional gel electrophoresis of immunoprecipitates. Results of this study provide immunologic evidence for homology between I-A and I-E antigens, and hence for gene duplication within the I region.

Bhattacharya, A., Dorf, M. E. and *Springer, T. A.*

The Journal of Immunology 127(6):2488-2495, 1981.

Other support: U. S. Public Health Service.

From the Laboratory of Membrane Immunochemistry, Sidney Farber Cancer Institute, and the Department of Pathology, Harvard Medical School, Boston.

NATURAL KILLER ACTIVITY IN THE PERITONEAL EXUDATES OF MICE INFECTED WITH *LISTERIA MONOCYTOGENES*: CHARACTERIZATION OF THE NATURAL KILLER CELLS BY USING A MONOCLONAL RAT ANTI-MURINE MACROPHAGE ANTIBODY (Ml/70)

Natural killer (NK) cells are mononuclear cells of disputed lineage that kill certain destructive cells in the body. In the paper presented here it can be seen that infection with *Listeria monocytogenes* (LM) leads to the generation of NK activity in peritoneal exudates. Specifically, maximum expression of NK activity first occurred on day two and remained high until day six after initial exposure to LM. When nylon wool nonadherent peritoneal exudate cells were examined by a single-cell cytotoxicity assay, the number of cells binding to YAC-1 target cells increased after infection as did their individual lytic capacity. A monoclonal rat anti-murine macrophage antibody (Ml/70), previously shown to recognize human NK cells, can be used also as a marker for murine NK cells. Utilizing Ml/70 and the fluorescence-activated cell sorter, selection of Ml/70-labeled mononuclear cells led to the enrichment of both NK and antibody-dependent cellular cytotoxicity. These Ml/70-positive cells had a distinctive morphology and contained granules on Wright-Giemsa staining. They were not phagocytic, did not contain nonspecific esterase, and lacked surface I-A^k, IgM determinants, complement receptors, and high levels of Thy 1.2.

Holmberg, L. A., *Springer, T. A.* and Ault, K. A.

The Journal of Immunology 127(5):1792-1799, 1981.

Other support: National Institutes of Health.

From the Department of Pathology, Harvard Medical School, Boston.

MACROPHAGE DIFFERENTIATION ANTIGENS: MARKERS OF MACROPHAGE SUBPOPULATIONS AND TISSUE LOCALIZATION

The characteristics of four distinct antigens which are present on macrophages, but not lymphocytes, are reviewed in this paper. In addition, two applications of anti-macrophage monoclonal reagents—their use in phenotyping macrophage subpopulations and in identification of macrophages in tissue sections—are described here. The major sections of this paper are devoted to MAC-1 ANTIGEN, IMMUNO-ADSORBENT-CELL HYBRIDIZATION CASCADES, MAC-2, 3 AND 4 ANTI-

GENS, and IDENTIFICATION OF MACROPHAGES IN TISSUE SECTIONS BY INDIRECT IMMUNOFLUORESCENCE. Overall, four macrophage antigens with distinct Mr and tissue distribution are identified in this work. Two of these, Mac-1 and Mac-3, are synthesized by all macrophage subpopulations examined thus far. However, Mac-2 seems to be preferentially associated with thioglycollate-elicited peritoneal macrophages. Ia antigens show a different pattern of expression. Therefore, macrophages can be defined into subsets with distinct antigenic phenotypes, as is the case for lymphocytes.

Springer, T. A. and Ho, M-K.

In: Mitchell, M. S. and Oettgen, H. F. (eds.): *Hybridomas in Cancer Diagnosis and Treatment*, New York: Raven Press, 1981, pp. 35-46.

Other support: U. S. Public Health Service.

From the Department of Pathology, Harvard Medical School, Boston.

RAT ANTI-MOUSE MACROPHAGE MONOCLONAL ANTIBODIES AND THEIR USE IN IMMUNOFLUORESCENT STUDIES OF MACROPHAGES IN TISSUE SECTIONS

The characteristics of five monoclonal antibodies (MAb) to macrophage antigens are summarized in this paper, which also contains a description of the use of one of these MAb for the localization of macrophages in frozen spleen sections. Of the five rat monoclonal antibodies to mouse macrophage surface antigens that were developed in the authors' laboratory by the myeloma-spleen cell hybrid technique of Kohler and Milstein, MI/70, which recognizes a phagocyte-specific antigen, Mac-1, is the most extensively studied. Two other antibodies, M3/31 and M3/38, precipitate a polypeptide termed Mac-2, which is also characterized here. In the related study, anti-Mac-1 was used to stain macrophages in spleen sections because Mac-1 seems to be expressed on macrophages irrespective of their state of differentiation and activation. To allow alignment of areas with Mac-1$^+$ cells with T-dependent areas of the spleen, adjacent sections were stained with M5/49, an anti-Thy-1 MAb. Results showed that T lymphocytes in the periarteriolar lymphatic sheath are intensely stained by anti-Thy-1 MAb. In contrast, few, if any, Mac-1$^+$ cells can be seen in these T-dependent areas. The simple method for the localization of macrophages described in this publication can be easily extended to other studies, especially with anti-Mac 1, which stains all macrophage subpopulations examined so far. In view of the vast body of information that can be gained from anatomical localization of lymphocyte subpopulations, these studies should provide much insight into the function, differentiation, and ontogeny of macrophages.

Ho, M-K. and *Springer, T. A.*

In: Hammerling, U., Hammerling, G. and Kearney, J. (eds.): *Monoclonal antibodies and T cell hybridomas*, New York: Elsevier/North Holland Biomedical Press, 1981, pp. 53-61.

Other support: U. S. Public Health Service.

From the Laboratory of Membrane Immunochemistry, Sidney Farber Cancer Institute, Harvard Medical School, Boston.

MAC-2, A NOVEL 32,000 M_r MOUSE MACROPHAGE SUBPOPULATION-SPECIFIC ANTIGEN DEFINED BY MONOCLONAL ANTIBODIES

The biochemical characterization and cell distribution of a 32,000 M_r antigen, termed Mac-2, are presented in this paper. Mac-2 is synthesized by and expressed on the surface of thioglycollate-elicited macrophages as shown by [^{35}S]-methionine and ^{125}I labeling. Unelicited peritoneal macrophages and macrophages elicited by protease peptone, Con A, LPS, and *Listeria monocytogenes* are either only weakly positive or negative. Therefore, Mac-2 expression is induced only by strong inflammatory stimuli and appears specific for mononuclear phagocyte subpopulations in a distinct stage of differentiation. Results of saturation binding experiments show that thioglycollate-elicited macrophages express 1.7×10^5 Mac-2 sites/cell. Thioglycollate-elicited macrophages are strongly absorptive for ^{125}I-labeled M3/38 MAb. Cell suspensions from spleen, bone marrow, thymus, and peripheral lymph node are > 99% Mac-2 negative by immunofluorescent flow cytometry. In contrast, thioglycollate-elicited macrophages are > 96% strongly positive for Mac-2. SDS-PAGE of [^{35}S]-methionine-labeled Mac-2 shows that thioglycollate-elicited macrophages synthesize 10- to 30-fold more Mac-2 than other peritoneal macrophage subpopulations, whereas all types of peritoneal macrophages synthesize and express on their surfaces similar amounts of the Mac-1 antigen. Mac-2 antigen is, therefore, induced in macrophages only in response to specific differentiative signals.

Ho, M-K. and *Springer, T. A.*

The Journal of Immunology 128(3):1221-1228, 1982.

Other support: U. S. Public Health Service.

From the Laboratory of Membrane Immunochemistry, Sidney Farber Cancer Institute, Boston.

ONTOGENY OF MURINE MACROPHAGES: FUNCTIONS RELATED TO ANTIGEN PRESENTATION

Immature macrophage function contributes to the increased susceptibility of neonates to infection. In this paper, the immaturity of neonatal macrophage function is dissected into antigen presentation and three different effector components: cytotoxicity, antigen uptake and catabolism, and the production of the lymphostimulatory molecule interleukin-1 (also called thymocyte mitogenic protein or lymphocyte-activating factor). The uptake and catabolism of ^{125}I-labeled *Listeria monocytogenes* were equivalent in macrophages from adult and neonatal mice. However, interactions between macrophages from neonates, heat-killed *Listeria* organisms, and immune T lymphocytes were impaired, and no cytocidal macrophages capable of killing tumor cells were generated. Previous studies with cells from adult mice had established that the development of cytocidal macrophages required Ia-bearing, antigen-presenting macrophages and histocompatibility at I-A between macrophages and T cells. To circumvent this requirement for antigen-presenting macrophages, an assay was used in which lymphokine was added directly to the macrophages from neonates. Strong cytocidal activity resulted. Thus, these studies showed that macrophages from neonates present antigen poorly but can acquire cytocidal function provided that the need

for antigen-presenting function is bypassed. Similar conclusions were reached for the secretion of interleukin-1. In essence, all the data presented here indicate that the impairment of a number of macrophage functions in the neonates is due to a reduced number of Ia-positive macrophages.

Lu, C. Y. and *Unanue, E. R.*

Infection and Immunity 36(1):169-175, 1982.

Other support: National Institutes of Health and the March of Dimes.

From the Department of Pathology, Harvard Medical School, Boston.

CONTROL OF MACROPHAGE Ia EXPRESSION IN NEONATAL MICE—ROLE OF A SPLENIC SUPPRESSOR CELL

As reported in this paper, the control of macrophage expression of I region-associated antigens (Ia) in neonatal mice was studied by comparing responses of neonatal and adult mice to immune vs. nonimmune stimuli. Adults generated peritoneal exudates rich in Ia-bearing macrophages in response to i.p. injection of live *Listeria monocytogenes*, *Listeria*-immune T cells, and heat-killed *Listeria*, or a soluble mediator termed macrophage Ia-recruiting factor (MIRF). Neonates failed to respond to these stimuli. In contrast, both neonates and adults generated Ia-negative peritoneal exudates when stimulated with thioglycollate. There are three major new points that came out of these studies: (1) neonatal mice not only have a defect in their basal number of Ia-positive phagocytes but also fail to respond to the immune stimuli that generate exudates enriched for these cells; (2) there is a suppressor system operating in the neonate capable of significantly dampening the recruitment of Ia-positive macrophages—this suppressor system is also operant in some adult tissues such as bone marrow and the peritoneal cavity; and (3) the suppressor mechanism involves, at least, the phagocyte system by way of an indomethacin- and aspirin-sensitive step. Overall, it appears that this phagocytic line autoregulates its surface expression of Ia in both neonatal and adult mice. Since this mechanism becomes particularly pointed during early development, it could contribute to the lack of immunity during ontogeny.

Snyder, D. S., Lu, C. Y. and *Unanue, E. R.*

The Journal of Immunology 128(3):1458-1465, 1982.

Other support: National Institutes of Health and the March of Dimes.

From the Department of Pathology, Harvard Medical School, Boston.

SPONTANEOUS T-CELL LYMPHOKINE PRODUCTION AND ENHANCED MACROPHAGE Ia EXPRESSION AND TUMORICIDAL ACTIVITY IN MRL-lpr MICE

Selected macrophage functions in MRL/Mp-lpr/lpr (MRL-lpr) mice were evaluated for this report. Specifically, three macrophage functions were studied in MRL-lpr mice with autoimmune lymphoproliferative disease: surface expression of I-region-associated (Ia) antigens, tumor cytotoxicity, and interleukin-1 (IL-1) production. MRL-lpr mice had a significantly increased representation of Ia-positive macrophages

in the peritoneal cavity, compared to all normal strains of mice. In order to study the basis of this increase, thymocytes or splenocytes from MRL-lpr mice were transplanted intraperitoneally into normal mice. Three days later the recipient mice had peritoneal exudates rich in Ia-positive macrophages. The cells which induced this response were T cells which elaborated a lymphokine responsible for the recruitment of Ia-positive macrophages. In previous studies from this laboratory using mice, lymphokine was secreted only following the interaction of immune T cells with antigen. The resident macrophages of MRL-lpr mice were activated and killed tumor cells if triggered by an interaction with bacterial products, even without the addition of lymphokines. Secretion of IL-1 was normal. Results indicate that the diseased MRL-lpr mice are characterized by (i) activated T cells that spontaneously secrete macrophage stimulatory molecules, and (ii) activated macrophages that show both an increased expression of Ia and lymphokine-independent triggering of tumoricidal activity.

Lu, C. Y. and *Unanue, E. R.*

Clinical Immunology and Immunopathology 25:213-222, 1982.

Other support: National Institutes of Health and the March of Dimes.

From the Department of Pathology, Harvard Medical School, Boston.

VII. *Epidemiology*

MORTALITY IN MIDDLE-AGED SMOKERS AND NONSMOKERS

The relation of cigarette smoking to mortality was assessed in an 11-year follow-up study of 4,004 men and women, 35-54 years of age, who responded to urging to have multiphasic health checkups. Accounting for 48 other characteristics, both individually and in combination, failed to eliminate the association of smoking with mortality from all causes or with mortality from coronary heart disease. The smoker-to-nonsmoker mortality ratios, crude and adjusted respectively, were 2.6 and 2.1 for all causes and 4.7 and 3.6 for coronary heart disease. This analysis did not support the counterhypothesis that the association of cigarette smoking with mortality is secondary to some underlying characteristic.

Friedman, G. D., Dales, L. G. and Ury, H. K.

The New England Journal of Medicine 300(5):213-217, 1979.

Other support: Kaiser Foundation Research Institute.

From the Department of Medical Methods Research, Kaiser-Permanente Medical Care Program, Oakland, CA.

CHARACTERISTICS OF SMOKING-DISCORDANT MONOZYGOTIC TWINS

The Kaiser-Permanente Twin Registry of Oakland, CA, contains over 8,000 pairs of twins who volunteered their participation in a program of medical research on twins. In 1977 and 1978 a large health questionnaire was mailed to all twins aged 18 years and over. After the first mailing in June 1977, a reminder postcard was sent in October 1977 to nonresponding same-sex twins. In this questionnaire, each twin was asked about his or her own smoking habits and those of his or her cotwin. In the study presented here, the smoking habits of the 33 cigarette smokers in the smoking-discordant monozygotic (MZ) female pairs were compared with those of the 205 other MZ female cigarette smokers. Large and statistically significant differences were noted in some measures of smoking intensity. Cigarette smokers who had an MZ nonsmoking cotwin tended to start smoking later and to smoke fewer cigarettes. This may explain, in part, the smaller difference in CHD occurrence between smokers and nonsmokers within smoking-discordant twins than between smokers and nonsmokers in the general population. Additional comparisons were also made between the discordant smokers and their nonsmoking cotwins. Results of these comparisons show that, with regard to coronary heart disease (CHD) risk factors, the discordant smokers were leaner and consumed more alcohol than their nonsmoking cotwins. While these traits are associated with a lower risk of CHD, smokers also tended to be less educated and reported less exercise and concern about physical fitness, consistent with higher risk. In conclusion, the data on a limited number of smoking-discordant female MZ twins suggest that, even with genetic identity, twins who differ in one characteristic may differ in other characteristics relevant to the outcome under consideration.

Friedman, G. D. et al.

In: Gedda, L., Parisi, P., and Nance, W. E. (eds.): *Twin Research 3: Epidemiological and Clinical Studies*, New York: Alan R. Liss, Inc., 1981, pp. 17-22.

Other support: National Heart, Lung and Blood Institute.

From the Department of Medical Methods Research and Department of Medicine, Kaiser-Permanente Medical Care Program, Oakland; University of California, Berkeley; and the Department of Epidemiology and International Health, University of California, San Francisco.

STRUCTURAL ANALYSIS OF SMOKING, ALCOHOL USE, AND PERSONALITY FACTORS IN MZ AND DZ TWIN PAIR RELATIONSHIPS

This study presents an extension of the testing of twin type comparability to a multivariate situation. Using data drawn from the Finnish Twin Cohort as the study population, smoking characteristics, alcohol use patterns, and personality factors of monozygotic (MZ) and dizygotic (DZ) twin pairs concordant and discordant for frequent heavy alcohol use were described. The effect of constraining covariance relationships in individuals of either twin type was tested to estimate comparability of pairwise structures. Also, the covariance relationships in individual twins were compared to those of singletons. For such analyses, the general model for the estimation of linear structural equation systems by maximum likelihood methods was applied to the Finnish Twin Cohort study data. Results showed that the differences between heavy-

and light-drinking individuals were most clear-cut in singletons. They differed in the mean smoking levels, and the extraversion and neuroticism scores. The same result was obtained in DZ pairs discordant for heavy alcohol use. In discordant MZ pairs, however, the smoking differences were observed but the personality factor differences were seen in the neuroticism and life dissatisfaction scales, suggesting that the basis of discordance in MZ twins may differ from that in DZ pairs and singletons. The proportion of discordant pairs was 29% in MZ and 37% in DZ pairs, less than the expected value. Multivariate analysis of differences in MZ and DZ discordant pairs confirmed the results from the univariate analyses, the relative significance of neuroticism in MZ pairs becoming weaker and statistically nonsignificant. Smoking, neuroticism, and life dissatisfaction, independent of genetic factors, seem to be indicators of processes leading to heavy alcohol use.

Langinvainio, H., Kaprio, J., Koskenvuo, M., and Tarkkonen, L. *(Rantasalo, I.)*

In: Gedda, L., Parisi, P. and Nance, W. E. (eds.): *Twin research 3: epidemiological and clinical studies*, New York: Alan R. Liss, Inc., 1981, pp. 23-35.

From the Department of Public Health Science, University of Helsinki, Helsinki.

CIGARETTE SMOKING, USE OF ALCOHOL, AND LEISURE-TIME PHYSICAL ACTIVITY AMONG SAME-SEXED ADULT MALE TWINS

The relationships of cigarette smoking, alcohol use and leisure-time physical activity among adult male twin participants in a Finnish population study are presented here. For this epidemiological study, questionnaire responses from 1,537 monozygotic (MZ) and 3,507 dizygotic (DZ) male pairs aged 18 and over were analyzed in terms of combinations of the three factors and the relationship of these factors to each other in relation to the twin pair situation. To do this, three factor analyses and an overall cluster analysis were carried out. Results of these tests showed that the physical activity factor means were almost constant with age, but there was a decrease with age in alcohol consumption; for the smoking factor, there was a steady increase with age until 50-54 years, after which a slight decrease occurred. The correlation coefficients between the factors in the whole series showed a high correlation between cigarette smoking and use of alcohol, and small negative correlations for physical activity and cigarette smoking and for physical activity and use of alcohol. In the cluster analysis, eight clusters were found to be stable in group-regroup situations with over 90% of members remaining in the same cluster from one analysis to another. As to twinship, both MZ and DZ twin pair members were in the same cluster much more often than expected, but the MZ-DZ overall difference was relatively small. The highest MZ/DZ ratios of observed to expected clustering rates were in two clusters: A) cluster no. 7, which had persons with a high mean degree of leisure-time physical activity; and B) the very small cluster no. 8, which had a very high mean alcohol use.

Kaprio, J., Koskenvuo, M. and Sarna, S. *(Rantasalo, I.)*

In: Gedda, L., Parisi, P. and Nance, W. E. (eds.): *Twin research 3: epidemiological and clinical studies*, New York: Alan R. Liss, Inc., 1981, pp. 37-48.

From the Department of Public Health Science, University of Helsinki, Helsinki.

CORONARY-PRONE BEHAVIOR IN ADULT SAME-SEXED MALE TWINS: AN EPIDEMIOLOGICAL STUDY

In this attempt to identify familial and environmental components of coronary-prone behavior patterns, the responses from 5,419 male twin pairs in the Finnish Twin Cohort to a 1975 questionnaire were investigated in several different ways. To begin with, the postal questionnaire study provided data on zygosity, smoking, alcohol use, leisure-time physical activity, weight, height, and drug usage. Psychosocial factors such as marital status, occupation and occupational history, changes of residence and employment, extroversion and lability, and type A behavior were also studied, as well as various symptoms and history of disease. Type A behavior pattern was measured by the rating scale developed by Bortner. Results of this study showed that the intraclass correlations were 0.251 for monozygotic (MZ) pairs and 0.052 for dizygotic (DZ) pairs. The heritability estimates were higher in younger than in older age groups, and the proportion of A-type concordant pairs also showed an age trend. While the proportion of MZ pairs among A-type concordant pairs was greater than among B-type concordant pairs, the difference was not statistically significant. In this study, an association in men between A-type behavior pattern and angina pectoris on the Rose questionnaire was found. Moreover, the discordant pair analysis presented here showed that there were some environmental factors clearly associated with coronary-prone behavior. As of now, A-type behavior has been shown to be an independent risk factor for different manifestations of coronary heart disease (CHD). Since some psychosocial factors, such as marital status and social class, which were found to correlate with A-type behavior in this study, are known to be associated with CHD in Finland, it seems reasonable that the relationship of A-type behavior, psychosocial factors, and CHD should be investigated further.

Koskenvuo, M., Kaprio, J., Langinvainio, H., Romo, M. and Sarna, S. *(Rantasalo, I.)*

In: Gedda, L., Parisi, P. and Nance, W. E. (eds.): *Twin research 3: epidemiological and clinical studies,* New York: Alan R. Liss, Inc., 1981, pp 139-148.

From the Department of Public Health Science, University of Helsinki, Helsinki.

FINNISH TWINS REARED APART: PRELIMINARY CHARACTERIZATION OF REARING ENVIRONMENT

This paper presents some characteristics of the rearing environment of 478 Finnish-speaking, adult, like-sexed twin pairs raised apart from the age of 10 or less. The Finnish Twin Cohort Study provided the raw data base for this study, and twinship was confirmed by a questionnaire study in 1975 that covered health-related items and standardized measures of morbidity. In addition to these questions, a number of other aspects were considered: whether the twin pair lived together and, if not, at what age separation had occurred. The present frequency of intrapair contact, birth order, and handedness were also investigated and questions directed to zygosity assessment were included. Later, during November 1979—January 1980, a new questionnaire on their childhood environment went out to the 478 twin pairs in the test group and three corresponding control groups, which were formed to assess which aspects of the rearing environment, personality factors, and childhood medical history of the study sample differed from those of twins of the same age and sex. Listings for this study are

given by age of separation (groups I-IV) and by birth year and sex. Results of this study showed that the intrapair correlation of rearing environment varied greatly as it appeared from variables such as age at separation, family members, school, friends, living place, intrapair contact frequency, and education and occupation of rearing parents. Moreover, the cause of separation, based on self-report, seemed to be fairly often associated with some psychosocial pathology. The separation of members of a twin pair may also mean intrapair selection. Further assessment and comparisons with singletons from the general population and with psychiatric outpatients are ongoing for this study.

Langinvainio, H. *et al. (Rantasalo, I.)*

In: Gedda, L., Parisi, P. and Nance, W. E. (eds.): *Twin research 3: intelligence, personality, and development,* New York: Alan R. Liss, Inc., 1981, pp. 189-198.

From the Departments of Public Health Science and Psychiatry, University of Helsinki, Helsinki.

CANCER IN ADULT SAME-SEXED TWINS: A HISTORICAL COHORT STUDY

In this attempt to investigate the feasibility of utilizing the twin method as a case-control type of study, a historical record-linkage cohort study between the Finnish Twin Cohort Study and the Finnish Cancer Registry was carried out. The Finnish Twin Cohort was created in 1974 from the computerized Central Population Registry, while the Finnish Cancer Registry is a population-based, national registry in operation since 1953. It is considered to be rather complete with respect to incident cases of cancer in Finland. For this study, persons included in both registries were identified by comparing by computer the personal identification numbers (a 10-digit unique code assigned to each resident in Finland) of the two registries. The comparison covered the years 1967-1976. The twin record linkage yielded the observed numbers of cancers of different types. Age and sex-specific person-years at risk were calculated separately for the twin population and the singleton group. Also, person-years at risk were calculated for cotwins of cancer probands. Results of this study showed that the total cancer morbidity in the twin population was lower than expected for both men and women. The relative risk for all cancers was 0.77 for men and 0.72 for women. In the singleton population, the relative risk for men was slightly over unity. In this study, the ratio of the observed to expected cancer morbidity closely reflected the standardized (total) mortality ratios for the same calendar years, suggesting that the lower-than-expected cancer morbidity may have a background in common with the lower-than-expected mortality. Also, the low concordance rate found in this study suggests that it may be fruitful to study the environmental exposure of cancer-discordant MZ and DZ twins.

Kaprio, J. *et al. (Rantasalo, I.)*

In: Gedda, L., Parisi, P. and Nance, W. E. (eds,): *Twin research 3: epidemiological and clinical studies,* New York: Alan R. Liss, Inc., 1981, pp. 217-223.

From the Department of Public Health Science, University of Helsinki, and Finnish Cancer Registry, Helsinki.

SLEEP DISORDERS IN RELATION TO CORONARY HEART DISEASE

Evidence from an American study indicating a relationship between sleep time and mortality, including death from coronary heart disease (CHD), led to a Finnish Twin Cohort study on the relationship between sleep time and CHD. In the epidemiological paper presented here, the sleeping time distributions in the U.S.A. taken from the Kripke study were compared to data obtained in 1975 from 5,419 Finnish adult men, and age-standardized proportions were computed for men aged 30 and over. Results showed, first of all, that the proportion of men sleeping 9-10 hours or more is higher in Finland than in the United States. They also showed that short (less than six hours) or long (more than 10 hours) sleepers had significantly more complaints relating to CHD than those who slept for seven-eight hours per night. Shortened sleep was especially related to angina pectoris and pain of possible infarction, and correlated with aging, poor sleep quality and/or Type A behavior pattern score. Long sleep, on the other hand, was correlated with good subjective sleep quality, but this group had the highest incidence of diagnosed myocardial infarction. This relationship held after statistically controlling for many possible confounders such as hypertension, drug and alcohol use, smoking and Type A behavior pattern. In addition, the cardiovascular physiology and pathophysiology of sleep is reviewed here and the relationship of some specific sleep disorders to CHD is discussed.

Partinen, M. *et al. (Rantasalo, I.)*

Acta Medica Scandinavica (Suppl) 660:69-83, 1982.

Other support: Finnish Foundation for Cardiovascular Research.

From the Departments of Neurology, Physiology and Public Health Science, University of Helsinki, Helsinki.

MULTIVARIATE LOGIT ANALYSIS OF CONCORDANCE RATIOS FOR QUALITATIVE TRAITS IN TWIN STUDIES

This statistical paper presents a new approach for the analysis of a certain type of twin data. The model that is applied in this paper, the logit model, is analogous to the widely used log-linear model for contingency table analysis. This model, which permits testing of interaction effects before estimating the main effects of the study variables, may also be easily extended to four-way or even more complex tables, though the testing procedures and sequential hypothesis testing becomes increasingly demanding. In the example used for this study, data from a cross-national study of cigarette smoking among adult twin pairs in two countries was used. The multivariate assessment of genetic factors in relation to other factors was carried out by logit analysis of concordance ratios by analyzing three variables, zygosity, sex, and country, at the same time. Thus, the effect of sex and country on zygosity in the smoking trait could be controlled. For cigarette smoking, the present results indicate that in both countries the zygosity effect is significant, and independent of country and sex. Although not presented here, the results held for both current and ever cigarette smoking as well as for smoking over one pack a day either currently or ever. For the heavy smokers, the zygosity effect had a significant interaction with sex. A significant zygosity effect implies greater concordance for the trait among MZ than DZ pairs and is due to the identical genome and probably greater common environment of MZ twin pairs.

Kaprio, J., Sarna, S., and Koskenvuo, M. *(Rantasalo, I.)*

Acta Geneticae et Medicae Gemellologiae 30:267-274, 1981.

From the Department of Public Health Science, University of Helsinki, Helsinki.

Active Projects

Following is a list of the principal investigators, or institutions, of projects under way or activated in the period since the previous Report, together with the respective project titles. Completed projects are listed in a later section.

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
JOHN J. ALBERS, PH.D., *Research Associate Professor of Medicine,* University of Washington School of Medicine, Seattle.	High density lipoprotein quantitation
HARRY N. ANTONIADES, PH.D., *Professor of Biochemistry,* Harvard University School of Public Health, Boston.	Human platelet-derived growth factor (PDGF): relationship to human atherosclerosis
THOMAS M. AUNE, PH.D., *Adjunct in Immunology,* The Jewish Hospital of St. Louis.	Interferon—activation of suppressor T cell pathways
BERNARD M. BABIOR, M.D., PH.D., *Professor of Medicine,* New England Medical Center Hospital, Boston.	Studies on the mechanism of activation of the respiratory burst in neutrophils
LESLIE BAER, M.D., *Associate Professor of Medicine,* Columbia University College of Physicians & Surgeons, New York.	Cigarette smoking in normotensive and hypertensive subjects: blood pressure, renin, aldosterone and catecholamine responses
BEA J. van den BERG, M.D., *Research Pediatrician, Adjunct Professor in Biostatistics.* University of California School of Public Health, Oakland.	Pulmonary function tests in adolescents and their parents—an epidemiologic approach
RICHARD J. BING, M.D., *Professor of Medicine (emeritus),* University of Southern California School of Medicine, Los Angeles; *Visiting Associate in Biomedical Engineering,* California Institute of Technology; *Director of Cardiology and Intramural Medicine,* Huntington Memorial Hospital, Pasadena, CA.	Cholesterol inhibition and carbon monoxide and atherosclerosis Lipoproteins and the arterial wall
DEBAJIT K. BISWAS, PH.D., D.Sc., *Associate Professor of Oral Biology,* Laboratory of Pharmacology, Harvard School of Dental Medicine, Boston.	Effects of nicotine and benzo(a)pyrene on hormone production
IRA B. BLACK, M.D., *Professor and Chief, Division of Developmental Neurology,* Cornell University Medical College, New York.	Nicotine and neuronal development
PHYLLIS B. BLAIR, PH.D., *Professor of Immunology,* University of California, Berkeley.	Regulation of natural killer cell activity

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
J. MARK BRAUGHLER, PH.D., *Assistant Professor of Pharmacology,* Northeastern Ohio Universities College of Medicine, Rootstown.	The alteration of guanylate cyclase by nitric oxide
EDWARD BRESNICK, PH.D., *Professor and Chairman, Department of Biochemistry,* The University of Vermont College of Medicine, Burlington.	Expression of cytochrome P450c
REBECCA BRYSON, PH.D., *Associate Professor of Psychology,* San Diego State University, San Diego, CA.	Interactive effects of nicotine, testosterone and estradiol on weight change, food consumption and activity of male and female rats under high and low protein diets
VINCENZO BUONASSISI, M.D., *Associate Research Biologist,* The University of California at San Diego, La Jolla.	Heparan sulfate proteoglycans and blood homeostatic mechanisms
DAVID L. BUSBEE, PH.D., *Director, Genetics Center; Associate Professor of Biological Sciences,* North Texas State University, Denton.	The biochemical and physiological characteristics of a protein which specifically binds polycyclic aromatic hydrocarbons
EDWARD J. CAMPBELL, M.D., *Assistant Professor of Medicine,* Washington University School of Medicine, St. Louis.	Modulators of inflammatory cell proteolytic activity
WILLIAM A. CARTER, M.D., *Professor of Hematology and Medical Oncology,* Hahnemann Medical College, Philadelphia.	The interplay of immunosurveillance and interferon induction in tumorigenesis
ALBERT CASTRO, PH.D., *Director, Hormone Research Laboratory; Professor of Pathology and Medicine,* University of Miami School of Medicine, Miami, FL.	Nicotine in blood: detection by radioimmunoassay
FRANCIS C. CHAO, M.D., PH.D., *Senior Investigator,* Center for Blood Research, Boston.	Platelet activation and blood hypercoagulability
JAN F. CHLEBOWSKI, PH.D., *Assistant Professor of Biochemistry,* Medical College of Virginia, Richmond.	Calofimetric investigation of proteinase-α_2 macroglobulin interaction
CURT I. CIVIN, M.D., *Assistant Professor of Oncology & Pediatrics,* The Johns Hopkins Oncology Center, Baltimore.	Biochemistry and function of human granulopoietic antigens
CHARLES G. COCHRANE, M.D., *Member, Department of Immunopathology,* Scripps Clinic and Research Foundation, La Jolla, CA.	Mediation systems in inflammatory lung disease

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
BERNICE H. COHEN, PH.D., *Professor and Director, Human Genetics/Genetic Epidemiology Program*, The Johns Hopkins University School of Hygiene and Public Health, Baltimore.	Genetic-epidemiologic characteristics of smokers and nonsmokers
ROBERT W. COLMAN, M.D., *Professor of Medicine*, Temple University School of Medicine, Philadelphia.	Initiation of plasma coagulation and kinin forming systems in man
GIDON CZAPSKI, M.SC., PH.D., *Professor of Physical Chemistry*, The Hebrew University, Jerusalem, Israel.	On the toxicity of oxygen and superoxide ion: is superoxide toxic?
ROBERT ECHT, PH.D., *Professor of Anatomy*, Michigan State University, East Lansing.	The effects of hypoxic hypoxia, carbon monoxide and treatments influencing hypoxia toxicity on endocrine-like cells in airways of young rabbits and rabbit fetuses.
CARLTON K. ERICKSON, PH.D., *Professor of Pharmacology*, The University of Texas College of Pharmacy, Austin.	Blood-brain monitoring of sustained nicotine levels in rats
V. GENE ERWIN, PH.D., *Professor of Pharmacology; Dean*, University of Colorado School of Pharmacy, Boulder.	Actions of nicotine on isolated perfused mouse brain
	Effects of nicotine on neuropeptide secrection by intact mouse brain, a pharmacogenetic study
ALVAN R. FEINSTEIN, M.D., *Professor of Medicine & Epidemiology*, Yale University School of Medicine, New Haven, CT.	Smoking, detection bias and primary lung cancer
JOSEPH D. FELDMAN, M.D., *Immunopathologist*, Scripps Clinic and Research Foundation, La Jolla, CA.	Effects of aging and lipids on the immune system
THOMAS H. FINLAY, PH.D., *Associate Professor of Obstetrics and Gynecology*, New York University Medical Center, New York.	Structure, properties and regulation of mouse plasma protease inhibitors
BIRGITTA FLODERUS-MYRRHED, PH.D., *Assistant Professor of Environmental Hygiene*, The Karolinska Institute, Stockholm.	Epidemiologic research on the Swedish twin registries
JUDITH ANN FOSTER, PH.D., *Professor and Chairperson, Department of Biology*, Sycracuse University, Syracuse, NY.	Involvement of elastin in lung disease
JACK W. FRANKEL, PH.D., *Consultant in Medical Research*, Veterans Administration Medical Center, Bay Pines, FL.	Smoking and lung cancer: diagnostic test to identify persons at high risk
ALLAN P. FREEDMAN, M.D., *Assistant Professor of Medicine*, Hahnemann Medical College, Philadelphia.	The effect of cigarette smoking on the alveolar clearance rate of inert dust particles in the human lung

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
AARON E. FREEMAN, PH.D., *Staff Scientist*, California Biomedical Research Foundation, La Jolla, CA.	Rodent and human lung epithelial cell culture as a tool for carcinogenesis research *in vitro*
	Epithelial cell carcinogenesis *in vitro*
KJELL FUXE, M.D., *Professor of Histology*, The Karolinska Institute, Stockholm.	Nicotine, catecholamines and neuroendocrine functions
MORTON GALDSTON, M.D., *Associate Professor of Medicine*, New York University Medical Center, New York.	Biochemical basis of predisposition to chronic obstructive pulmonary disease
MICHAEL C. GEOKAS, M.D., PH.D., *Professor and Vice-Chairman, Department of Medicine*, University of California School of Medicine, Davis.	Circulating pancreatic elastase 2 and emphysema in man
TERESA GESSNER, PH.D., *Associate Cancer Research Scientist*, Health Research, Inc., Roswell Park Division, Buffalo.	Pharmacogenetics of conjugations and lung cancer risk
JACQUES E. GIELEN, PH.D., *Associate Professor, Laboratory of Medical Chemistry, Toxicology and Hygiene*, Institute of Pathology, University of Liège, Liège, Belgium.	Modulation of aryl hydrocarbon hydroxylase and epoxide hydratase in animal tissues and in cell culture by cigarette smoke condensate and other chemicals
	Biochemical mechanism(s) and qualitative and quantitative consequences of benzo(α)pyrene metabolism
GABRIEL C. GODMAN, M.D., *Professor of Pathology*, Columbia University College of Physicians & Surgeons, New York.	Cytoskeletal organization of the endothelial cell in regulation of shape contractility and surface movement
WARREN M. GOLD, M.D., *Professor of Medicine*, Cardiovascular Research Institute, University of California, San Francisco.	Effect of ozone on airway mast cells
SIDNEY GOLDFISCHER, M.D., *Professor of Pathology*, Albert Einstein College of Medicine, The Bronx, NY.	Extracellular matrix-cytochemistry and ultrastructure
MAURICE GREEN, M.D., *Director, Institute for Molecular Virology*, St. Louis University Medical Center, St. Louis.	Amplification of human adenovirus transformation proteins in prokaryotic and eukaryotic cells
MARK I. GREENE, PH.D., *Associate Professor of Pathology*, Harvard Medical School, Boston.	Suppressor cells in syngeneic tumor immunity
HIRA L. GURTOO, D.V.M., M.V. Sc., PH.D., *Associate Cancer Research Scientist, Department of Experimental Therapeutics*, Roswell Park Memorial Institute, Buffalo.	Role of genetics and polyaromatic hydrocarbon metabolism in human lung cancer

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
NOBUYOSHI HAGINO, M.D., PH.D., *Professor of Anatomy*, University of Texas Health Science Center, San Antonio.	Nicotinic receptors of LHRH axon terminals in the median eminence
CAROLINE B. HALL, M.D., *Associate Professor of Pediatrics and Medicine*, University of Rochester School of Medicine, Rochester, NY.	Interrelationship of infectious lower respiratory tract disease in infancy, and host and environmental factors to later development of chronic lung disease
LINDA M. HALL, PH.D., *Associate Professor of Genetics and Neuroscience*, Albert Einstein College of Medicine of Yeshiva University, The Bronx, NY.	Genetic differences in nicotine sensitivity in *Drosophila melanogaster* strains
PAUL HAMOSH, M.D., *Associate Professor of Physiology and Biophysics, and Medicine*, Georgetown University Schools of Medicine and Dentistry, Washington, D.C.	Cigarette smoke and lipoprotein remodeling by the lung
NORMAN W. HEIMSTRA, PH.D., *Professor of Psychology; Director, Human Factors Laboratory*, University of South Dakota, Vermillion.	Some behavioral aspects of smoking and smoking deprivation
HERBERT B. HERSCOWITZ, PH.D., *Associate Professor of Microbiology*, Georgetown University Schools of Medicine and Dentistry, Washington, D.C.	Effects of cigarette smoke exposure on developmental, cellular and molecular aspects of the immune response
ROBERT M. HOFFMAN, PH.D., *Assistant Professor of Pediatrics in Residence*, University of California School of Medicine, La Jolla.	Methionine dependence, methylation and organic transformation
	Regulation of cellular oncogenes
JEROME L. HOJNACKI, PH.D., *Assistant Professor of Biological Sciences*, University of Lowell, Lowell, MA.	Nicotine-induced changes in primate high density lipoproteins
WAYNE HOSS, PH.D., *Associate Professor*, Center for Brain Research, University of Rochester Medical Center, Rochester, NY.	Studies of nicotine interaction with blood cells
AARON JANOFF, PH.D., *Professor of Pathology*, Health Sciences Center, State University of New York at Stony Brook, Stony Brook.	Further studies on suppression of protease inhibition by cigarette smoke
	Immunologic assay of lung elastin degradation
MORRIS J. KARNOVSKY, M.B., B. CH., *Shattuck Professor of Pathological Anatomy*, Harvard Medical School, Boston.	The molecular basis of pulmonary surfactant secretion by type II pneumocytes: studies in intact cells and a cell free system
INGEGARD M. KEITH, PH.D., *Assistant Professor of Anatomy*, University of Wisconsin School of Veterinary Medicine, Madison.	Part I: Lung neuroendocrine cell innervation
	Part II: Transplacental effect of smoking on lung neuroendocrine cells in the neonate

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
ROBERT W. KREILICK, PH.D., *Professor of Chemistry*, University of Rochester, Rochester, NY.	Investigations of the interaction of nicotine with membranes
KLAUS E. KUETTNER, PH.D., *Professor and Chairman; Department of Biochemistry*, Rush College of Health Sciences and Rush Medical College, Rush-Presbyterian-St. Luke's Medical Center, Chicago.	Local regulation of normal and pathologic invasion
	Regulation of proliferation of invasive cells
LAWRENCE L. KUPPER, PH.D., *Associate Professor of Biostatistics*, University of North Carolina School of Public Health, Chapel Hill.	Verification of a statistical age-period-cohort analysis of lung cancer
ABEL LAJTHA, PH.D., *Director*, New York State Research Institute for Neurochemistry and Drug Addiction, New York.	Genetic basis for nicotine response
DON LAPENAS, M.D., *Assistant Professor of Pathology*, University of Vermont College of Medicine, Burlington.	The association of inorganic dust deposition with pulmonary neoplasia in tobacco users
E. CLINTON LAWRENCE, M.D., *Assistant Professor of Medicine*, Baylor College of Medicine, Houston.	Effects of cigarette smoking on immunoglobulin production by human bronchial lymphocytes
PHILIP M. LE QUESNE, PH.D., D.SC., *Professor of Chemistry*, Northeastern University, Boston.	Assay of oxygenated sterols in human blood vessels—a feasibility study
FABIAN J. LIONETTI, PH.D., *Research Professor of Biochemistry*, Boston University School of Medicine, Boston.	Phagocyte mediated injury to tissues
GESINA L. LONGENECKER, PH.D., *Associate Professor of Pharmacology*, University of South Alabama College of Medicine, Mobile.	Studies of platelet and endothelial prostanoid production as possible cardiovascular risk indicators in smokers
RONALD B. LUFTIG, PH.D., *Professor of Microbiology and Immunology*, University of South Carolina School of Medicine, Columbia.	Interactions between RNA tumor viruses and chemical carcinogens
HENRY T. LYNCH, M.D., *Professor and Chairman, Department of Preventive Medicine and Public Health*, Creighton University School of Medicine, Omaha.	Genetic and biomarker studies of smoking-associated cancers
BRUCE A. MACHER, PH.D., *Assistant Professor of Pharmaceutical Chemistry*, University of California, San Francisco.	Chemistry and biology of complex carbohydrates
ALAN C. McLAUGHLIN, PH.D., *Biophysicist*, Brookhaven National Laboratory, Upton, NY.	Interaction of divalent cations with model and biological membranes

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
J. WISTER MEIGS, M.D., *Clinical Professor of Epidemiology; Director, Connecticut Cancer Epidemiology Unit,* Yale University School of Medicine, New Haven, CT.	Review of lung cancer in Connecticut, 1935-present
FERID MURAD, M.D., PH.D., *Professor of Medicine and Pharmacology,* Stanford University, and *Chief of Medicine,* Palo Alto V.A. Hospital, Stanford, CA.	Mechanism of nitric oxide activation of guanylate cyclase
	Role of cyclic GMP in smooth muscle relaxation
JAY A. NADEL, M.D., *Professor of Medicine, Physiology and Radiology,* Cardiovascular Research Institute, University of California, San Francisco.	Mechanisms of airway hyperirritability
DONALD J. NELSON, PH.D., *Associate Professor of Chemistry,* Clark University, Worcester, MA.	Spectroscopic studies of the interaction of cholinergic ligands with nicotinic receptor proteins
HAROLD H. NEWBALL, M.D., *Associate Professor of Medicine,* The Johns Hopkins University School of Medicine, Baltimore.	The role of proteases and antiproteases in pulmonary emphysema
FRANZ OESCH, PH.D., *Professor of Pharmacology; Head, Section on Biochemical Pharmacology,* University of Mainz, Mainz, West Germany.	Metabolic fate and toxicological significance of dihydrodiols derived from polycyclic aromatic hydrocarbons occurring in cigarette smoke
BENDICHT U. PAULI, D.V.M., *Associate Professor of Pathology,* Rush Presbyterian-St. Lukes Medical Center, Chicago.	Local regulation of tumor invasion by host-derived proteinase inhibitors
DENNIS R. PETERSEN, PH.D., *Assistant Professor of Pharmacology,* University of Colorado School of Pharmacy, Boulder.	Influences of genotype, sex and chronic cigarette smoking on nicotine and alcohol metabolism in mice
JULIA M. POLAK, D.Sc., M.D., *Senior Lecturer in Histopathology,* Royal Postgraduate Medical School, Hammersmith Hospital, London.	Investigation of the role of regulatory peptides in human lung disease
WILLIAM A. PRYOR, PH.D., *Boyd Professor of Chemistry,* Louisiana State University, Baton Rouge.	Free radical chemistry of cigarette smoke
ILARI RANTASALO, M.D., *Professor and Chairman, Department of Public Health Science,* University of Helsinki, Helsinki, Finland.	The Finnish Twin Cohort Follow-up Study
RONALD E. RASMUSSEN, PH.D., *Associate Adjunct Professor in Community and Environmental Medicine,* University of California College of Medicine, Irvine.	The role of cell-specific toxins in mouse lung carcinogenesis

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
EILEEN REMOLD-O'DONNELL, PH.D., *Principal Research Associate,* Harvard Medical School; *Investigator,* Center for Blood Research, Boston.	Purification and functional analysis of elastase from guinea pig macrophages
JOHN E. REPINE, M.D., *Assistant Director, Webb-Waring Lung Institute; Associate Professor of Medicine,* University of Colorado Health Sciences Center, Denver.	Basic mechanisms of lung injury from inhaled oxidants
HERBERT Y. REYNOLDS, M.D., *Professor of Medicine; Head, Pulmonary Section,* Yale University School of Medicine, New Haven, CT.	Respiratory secretions in pulmonary carcinoma: secretory component of immunoglobulin-A as an early marker of epithelial dysfunction
	Markers of epithelial cell dysfunction in respiratory secretions of smokers
VIRGINIA L. RICHMOND, PH.D., *Research Associate,* Pacific Northwest Research Foundation, Seattle.	Elastic fiber microfibrillar protein structure
PETER M. ROSS, PH.D., *Research Associate,* The Rockefeller University, New York.	DNA damage and selective maintenance of animal genes
UNA S. RYAN, PH.D., *Research Professor of Medicine,* University of Miami School of Medicine, Miami, FL.	Interactions of hormones with cells of the pulmonary vascular wall
B. V. RAMA SASTRY, D.Sc., PH.D., *Professor of Pharmacology,* Vanderbilt University School of Medicine, Nashville, TN.	Influence of nicotine on the release of acetylcholine in the human placenta and its implications on the fetal growth
ISHAIAHU SCHECHTER, PHD., *Senior Lecturer in Biochemistry,* The George S. Wise Faculty for Life Sciences, Tel Aviv University, Tel Aviv, Israel.	Effect of thiols and disulfides on cholesterol metabolism
GERALD SHKLAR, D.D.S., *Charles A. Brackett Professor of Oral Pathology; Head, Department of Oral Medicine and Oral Pathology,* Harvard School of Dental Medicine, Boston.	Oral carcinogenesis, vitamin A and retinoids
ROBERT J. SKLAREW, PH.D., *Research Associate Professor of Pathology,* New York University Research Service, Goldwater Memorial Hospital, Roosevelt Island, New York.	Cytokinetics of heteroploid subpopulations by imaging
HANOCH SLOR, PH.D., *Associate Professor of Human Genetics,* Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel.	The use of specific antibodies to monitor the formation and removal of benzo-(α)pyrene adducts from DNA of damaged human cells
DENNIS M. SMITH, PH.D., *Assistant Professor of Biological Sciences,* Wellesley College, Wellesley, MA.	Autonomic control of pulmonary surfactant in the adult lung

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
TIMOTHY A. SPRINGER, PH.D., *Assistant Professor of Pathology*, Harvard Medical School, Boston.	Studies of macrophage subpopulations and differentiation using monoclonal antibodies
ERIC J. STANBRIDGE, PH.D., *Associate Professor of Microbiology*, University of California, Irvine.	Transfer of specific individual human chromosomes to recipient cells
DANIEL STEINBERG, M.D., PH.D., *Professor of Medicine; Head, Division of Metabolic Disease*, The University of California at San Diego, La Jolla.	Arterial degradation of low density lipoproteins *in vivo*
THOMAS P. STOSSEL, M.D., *Chief, Medical Oncology Unit*, Massachusetts General Hospital, Boston.	Functional anatomy of the lung macrophage
D. LANSING TAYLOR, PH.D., *Associate Professor of Biology*, Harvard University, Cambridge, MA.	Chemotaxis of macrophages
JAMES TRAVIS, PH.D., *Professor of Biochemistry*, The University of Georgia, Athens.	Proteolytic enzymes and inhibitors in emphysema
EMIL R. UNANUE, M.D., *Mallinckrodt Professor of Immunopathology*, Harvard Medical School, Boston.	Physiopathology of normal and activated macrophages
STEPHEN F. VATNER, M.D., *Associate Professor of Medicine*, Harvard Medical School, New England Regional primate Research Center, Southboro. MA.; *Associate in Medicine*, Peter Bent Brigham Hospital, Boston.	Direct effects of nicotine on brain circulation
PETER N. WALSH, PH.D., *Professor of Medicine*, Temple University School of Medicine, Philadelphia.	Interaction of platelets with coagulation factors IX and X
GEORGE WEINBAUM, PH.D., *Bioscientist, Pulmonary Disease Section*, Albert Einstein Medical Center, Philadelphia.	Bronchioalveolar lavage of human smokers and nonsmokers: studies on cell chemotaxis, enzyme release and cellular ultrastructure
I. BERNARD WEINSTEIN, M.D., *Professor of Medicine and Environmental Sciences*, Columbia University, New York.	Development of monoclonal antibodies to carcinogen-DNA adducts
SIGMUND A. WEITZMAN, M.D., *Assistant Professor of Medicine*, Hematology—Oncology Unit, Massachusetts General Hospital, Boston.	Studies of phagocyte—induced mutation
AKE WENNMALM, M.D., *Associate Professor of Clinical Physiology at Karolinska Institute*, Huddinge Hospital, Huddinge, Sweden.	Nicotine as inhibitor of prostaglandin formation: localization of the inhibitory step and characterization of the cardiovascular implications
JOHN T. WILSON, PH.D., *Assistant Professor of Cell and Molecular Biology*, Medical College of Georgia, Augusta.	The isolation and expression of human α-1-antitrypsin gene sequences through molecular cloning

PRINCIPAL INVESTIGATOR OR INSTITUTION	PROJECT TITLE
ALVIN WINTERS, PH.D., *Assistant Professor of Medical Microbiology*, Veterans Administration Medical Center, Bay Pines, FL.	Effects of smoking on the inherent interferon levels in control and cancer patients: a pilot study
BRUCE A. WODA, M.D., *Associate Professor of Pathology*, University of Massachusetts, Worcester.	Cell surface membranes, integral membrane proteins and cytoskeleton in lymphocytes from: (1) young and old rats; (2) spontaneous rat lymphomas
STANLEY YACHNIN, M.D., *Professor of Medicine and Chief, Section of Hematology/Oncology*, The University of Chicago Medical Center, Chicago.	Models for the pathogenesis of atherosclerosis: A) biological effects of oxygenated sterol compounds; B) mevalonic acid and cholesterol biosynthesis and the biosynthesis and regulation of cell growth

Completed Projects

Following is a list of the principal investigators, or institutions, of projects that have been completed prior to the period covered in this Report. Several of the individuals named are deceased. The titles and affiliations listed are those in effect at the time the work was completed.

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